Original paper

Evaluation of the cytogenetic profile in patients with acute leukaemia

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Acute leukaemia (AL) is a heterogeneous neoplastic disease that occurs by the growth of abnormal lymphoid and myeloid cells in the bone marrow and blood leading to acute myeloid leukaemia (AML) and acute lymphocytic leukaemia (ALL). Conventional cytogenetics is a characteristic technique to hunch chromosomal abnormalities, it helps in the diagnosis and therapeutic approach of the disease by the molecular cytogenetics technique of fluorescence in situ hybridization (FISH). Chromosomal abnormalities in AL are performed by karyotyping to confirm specific chromosomal abnormalities using FISH.

The descriptive study included 42 clinically diagnosed AL patients. Karyotyping analysis was performed using the standard Giemsa banding procedure. To confirm specific chromosomal abnormalities and all culture failure (CF) cases, FISH was done. Among 42 cases, 29 (69.4%) males and 13 (30.9%) females, AML comprised 22 (52.38%) cases, ALL 14 (33.33%) cases, and AL 6 (14.2%) cases. Normal karyotype was found in 18 (42.85%), abnormal karyotype in 16 (39.09%), and 8 (19.09%) were CF. Specific abnormalities of t(15;17), hyperdiploidy; t(3;3) with monosomy 7 in; del(9q22); del(2p); del(17p); del(Xq); 1~2 dmin; der(3); +11, +13 and composite karyotype. Hypodiploidy was strongly associated with AL, which signifies the loss of chromosomes causing potential risk.

Composite karyotype, rare t(3;3) double minutes, +11,+13, del(9q), and del(Xq) were the novel findings reported in the South Canara region of Karnataka. Despite other molecular techniques, conventional cytogenetics remains the baseline in the diagnosis of malignancies.

Key words: acute leukaemia, acute myeloid leukaemia, acute lymphocytic leukaemia, karyotyping, fluorescence in situ hybridization.

Introduction

Acute leukaemia (AL) is a neoplastic disease that occurs by the growth of abnormal lymphoid and myeloid cells. Acute leukaemia is also responsible for reducing the quality of life, leading to most cancer-related deaths in populations [1]. Acute myeloid leukaemia (AML) is a haematopoietic disorder characterized by abnormal myeloblasts infiltrating the normal bone marrow cells leading to the neoplasm [2]. Acute lymphocytic leukaemia (ALL) is a malignant disease that arises from several cooperative genetic mutations in a single B- or T-lymphoid progenitor, leading to altered blast cell proliferation, survival, maturation, and eventually lethal accumulation of leukemic cells [3]. It is widely associated with a broad spectrum of cytogenetic abnormalities more than any other malignancies in cancer.

Acute leukaemia consisting of non-random chromosomal abnormalities obtained from conventional cytogenetics in both AML and ALL has a clinical impact. The chromosomal aberrations associated with specific genes play a vital role in leukemogenesis identified by molecular studies [4]. Ploidy is a common chromosomal abnormality with loss and gain of chromosomes leading to hypodiploidy and hyperdiploidy associated with favourable outcomes in acute leukaemia [5]. Aggregation of clonal myeloid cells at the promyelocytic stage of differentiation leads to acute promyelocytic leukaemia (APL/AML M3), a subtype of AML.

Conventional cytogenetics plays a vital role in ruling out chromosomal abnormalities, which helps in diagnosis and in providing a therapeutic approach to the disease by karyotyping and fluorescence in situ hybridization (FISH) methods. Globally, many studies have been reported on cytogenetics in acute leukaemia, but very few reports are represented in the Indian population. Hence, the study's main aim was to detect the chromosomal abnormalities in AL by karyotyping and confirm the specific chromosomal abnormalities using FISH in the South Canara region of Karnataka.

Material and methods

This descriptive study was carried out on 42 clinically diagnosed AL patients. Their age ranged 1–88 years, referred to the KSHEMA Centre for Genetic Services (KS Hegde Medical Academy, Mangalore, Karnataka, India) for cytogenetic analysis. Written informed consent was obtaianed from all the patients and ethical approval was granted from the central Ethics Committee of the NITTE (deemed to be a university). Clinically diagnosed AL patients were included; patients associated with other haematological malignancies and not willing to participate were excluded from the study. Fluorescence in situ hybridization analysis was carried out to confirm the chromosomal abnormalities in culture failure (CF) cases and specific chromosomal abnormalities. For karyotyping and FISH, 2 ml peripheral venous blood or bone marrow in a sodium heparin vacutainer was collected from each patient.

Karyotyping

Conventional karyotype analysis was performed using the standard Giemsa banding procedure with slight modification [6]. Unstimulated 24-hour culture was initiated using 5 ml of Marrow Max media (Gibco, USA) with optimum sample volume based on the total white blood corpuscles (WBC) count. The culture flask was incubated for 24 hours at 37°C in a CO_2 incubator. Metaphases were arrested using 100 μl Colcemid and kept for incubation at 37°C in a CO_2 incubator for 20 minutes. The culture was transferred to a 15 ml centrifuge tube and then centrifuged at 2000 rpm for 10 minutes. The supernatant was discarded, and 10 ml of 0.075 M KCL was added as a hypotonic solution to increase the cell volume for 20 minutes at 37°C.

After hypotonic treatment, 2 ml of fixative, methanol, and acetic acid (Carnoy's fixative) in the ratio of 3 : 1 was added slowly drop-wise from the sides of the tube and centrifuged at 2000 rpm for 10 minutes. After centrifugation, the supernatant was discarded, and 10 ml of fixative was added. The tube was kept in the refrigerator for 30 minutes and centrifuged again. The supernatant was discarded, and the cell pellet was washed with fixative 3 times to obtain the clear pellet. The cell pellet obtained was dropped on pre-chilled slides and dried on a hotplate at 45°C and kept for ageing overnight at 60°C in a hot air oven. The next day, the slides were treated with trypsin (1 : 250) solution and stained in 1% Giemsa solution. A minimum of 20 well-spread metaphases with excellent band resolution was analysed and captured using a BX53 Olympus microscope. Three spreads with consistent chromosomal abnormalities were considered as abnormal karyotype. Analysis was performed using the GenASIs software (Version 8.1.1). Karyotypes were interpreted according to the International System for Human Cytogenetic Nomenclature –2013 [7].

Fluorescence in situ hybridization analysis

The fixed cells were dropped onto the slides and incubated for 2 minutes at 45°C on a hot plate, followed by immersing the slides in sodium saline citrate (SSC) for 5 minutes. Dehydration was carried out with different grades of alcohols (70%, 85%, and 100%). Then 10 μl of the probe (Cytocell, UK) was applied and covered with a coverslip. The sam-

ple and the probe were denatured at 75°C for 5 minutes, followed by overnight hybridization at 37°C in a Thermo Brite Denaturation/Hybridization system. The next day post hybridization was carried out using 0.4 × SSC (pH 7.0) in a water bath for 20 seconds at 70 ±1°C and then in 2 × SSC/0.5% at room temperature for 10 seconds. Then the slides were dried manually and counterstained using 10 μl of 4,6-diamino-2-phenylindole (DAPI). For each case, a total of 100 interphase nuclei and available metaphases were scored under oil immersion lens for the presence of signals using different filters in an Olympus BX-53 microscope equipped with DAPI, fluorescein isothiocyanate (FITC), and sulforhodamine 101 acid chloride red filters. The observed signals were captured and analysed using FISH view image acquisition (Genetic, Applied Spectral Imaging) software.

Fluorescence in situ hybridization was done on the following probes

The acute myeloid leukaemia panel comprised mixed lineage leukaemia (MLL) (11q23), acute myeloid leukaemia-eight twenty-one (AML/ETO) t(8;21), promyelocytic leukaemia/retinoic acid receptor α (PML/RARα –) t(15;17), core binding factor β (16), and t(16;16) probes.

The acute lymphocytic leukaemia panel comprised breakpoint cluster region-abelson murine leukaemia 1 (BCR-ABL1) t(9;22) and MLL (11q23) probes.

The expected signal pattern of these probes in normal cell lines should appear as discrete 2 red and 2 green (2R, 2G) for each homologue. In abnormal, AML/ETO t(8;21), PML/RARα t(15;17), and BCR-ABL1 t(9;22) cell lines, there should be 2 yellow signals in addition to the red and green signals of normal chromosomes (1R, 1G, 2Y) of each probe, respectively. Mixed lineage leukaemia (11q23) with 2 red and 2 green confirms positive for MLL break apart.

Results

Among 42 samples collected, 29 (69.4%) males and 13 (30.9%) females had an M/F ratio of 2.2 : 1. The cytogenetic findings of 42 AL patient's clinical and biological characteristics with frequency rate were mentioned (Table I, II). The mean and standard deviation of the patient's age was 39.07 ±22.22 years. The age-wise distribution of AL cases was shown (Fig. 1). Acute myeloid leukaemia was observed in 22 (52.38%), ALL were in 14 (33.33%), and 6 (14.2%) cases showed other forms of leukaemia. The incidence of AML in the present study population was predom-

Table I. Overall cytogenetic findings of 42 acute leukaemia patients

Karyotype	No. of Cases	Percentage (%)	Gender (M/F)	Age (years) Median (range)	WBC (109/l) Median (range)	Lymphocyte (%, 109/l) Median (range)
Total cases	42	100	29/13	39 (1–88)	35 (0.1–99)	69.5 (1.6–99.9)
Normal karyotype	18	42.85*	12/6	42.5 (13–67)	26.8 (0.3–99)	75.3 (1.6–99.9)
Abnormal karyotype	16	39.09*	10/6	44 (1–88)	14.9 (0.3–78)	67.45 (2–99.9)
Culture failure	8	19.09*	7/1	25.5 (5–70)	46.7 (0.1–99)	62.25 (1.7–99.9
Hyperdiploidy	2	12.5**	1/1	33 (23–43)	11.6 (8.2–15)	34.4 (2–66.8)
Hypodiploidy	6	37.5**	5/1	45 (1–88)	11.2 (5.7–78)	80.6 (26–91.6)
t(15;17)(q24;q21)	2	12.5**	0/2	29 (5–53)	13 (6–20)	80.5 (72–89)
t(3;3)(q22;q29)	1	6.25**	1/0	50	3.7	68.1
7 other	1	6.25**	1/0	50	3.7	68.1
+11	1	6.25**	1/0	41	48.9	15.5
+13	1	6.25**	1/0	34	34.3	71
del(9q22) alone	1	6.25**	1/0	84	12.4	75.6
del(2p) other	1	6.25**	0/1	50	78	57
del(17p) other	1	6.25**	0/1	50	78	57
del(Xq) other	1	6.25**	0/1	50	78	57
1~2 dmin other	1	6.25**	0/1	50	78	57
der(3) other	1	6.25**	0/1	50	78	57
Composite karyotype	1	6.25**	0/1	65	47	2.6

del – deletion
** Percentages calculated from the total 42 reported cases*
*** Percentages calculated from the 16 abnormal reported cases*

Table II. The baseline characteristics of the 42 acute leukaemia patients

Charasteristics	Value median (range)
Age (year)	41 (1–88)
Hb [g/dl]	9.9 (3.3–15.3)
ESR [mm/hr]	68.5 (15–140)
Total protein [g/dl]	6.9 (4.8–8.3)
Albumin [g/dl]	3.9 (2.5–4.9)
Globulin [g/dl]	3.3 (2.3–4.8)
Bilirubin direct	0.4 (0–2.4)
Bilirubin indirect	0.485 (0.05–3.1)
Bilirubin total [mg/dl]	0.915 (0.1–3.7)
SGOT [U/l]	33.5 (11.2–76)
SGPT [U/l]	29 (6.4–118)
ALP [U/l]	89 (70–181)
Chloride [mg/dl]	100 (1.1–133)
Sodium [mg/dl]	136 (118–177)
Potassium [mg/dl]	3.9 (2.8–6.3)
Blood urea [mg/dl]	24 (8–144)
TLC × 10^9/l	20.47 (0.28–148.13)
Creatinine	0.76 (0.2–2.3)
Platelet count × 10^9/l	143 (2–825)
ALC × 10^9/l	8.29 (0.02–147.98)
ANC × 10^9/l	10.84 (0.005–130.35)

ALC – absolute lymphocyte count, ALP – alkaline phosphatases, ANC – absolute neutrophil count, ESR – erythrocyte sedimentation rate, Hb – haemoglobulin, SGOT – serum glutamic-oxaloacetic transaminase, SGPT – serum glutamic-pyruvic transaminase, TLC – total leucocyte count

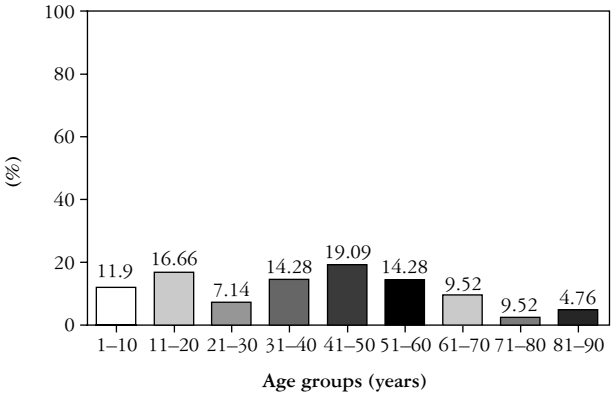

Fig. 1. Age distribution of acute leukaemia

inant with the age group > 50 years in 11 (50%) cases whereas in other age groups were 0–15 years in 1 (4.54%) case and 16–50 years in 10 (45.45%) cases, respectively. The incidence of ALL was predominant in the age group 16–50 years in 8 (57.1%) cases, whereas in other age groups: 0–15 years in 5 (35.71%) cases and > 50 years in 1 (7.14%) case, respectively. Other forms of AL were predominant in the age group 16–50 years in 5 (83.33%) cases and > 50 years in 1 (16.66%) case. The distributions of all abnormal cases are shown in Table III.

Conventional cytogenetics

A normal karyotype was observed in 18 (42.85%) cases, including 12 males and 6 females. Abnormal karyotype was observed in 16 (39.09%) cases, including 10 males and 6 females (Table II). The remaining 8 (19.09%) cases did not yield any

Table III. Chromosomal abnormalities of the acute leukaemia patients

Chromosomal abnormality	Karyotypes
Hypodiploidy	43~46,XY, –2,–3,–10,–16,–18,–21
	40~46,XY,–7,–3,–5,–10,–16,–17,–19,–21
	36~44,XY,–4,–5–10,–11,–11,–16,–20,+2mar
	43~45,XY,–4,–10,–14,–16,–18,–20,–21,–Y[cp8]
	40~45,XY,–7,–8,–9,–13,–14,–15,–16,–18,–Y
	39~44,XX,–1,–4,–7,–8,–12,–15,–20
Hyperdiploidy	48~58,XY,+1,+1,+4,+6,+7,+9,+10,+11,+14,+14,+15,+19,+20,+21, +21,+21,+21,+22,+22
	47,XX,–3,+12,+21\48,XX,+21
Composite karyotype	39~50,XX,+1,+2,+3,+9,+12,+20,+mar,–5,–7,–12,–15,–16,–18,–19, –20,X[cp7]
Translocation	45,XY, t(3;3)(q22;q29),–7
	46,XX, t(15;17)(q24;q21)
	46,XX, t(15;17)(q24;q21)
Trisomy	47,XY,+13
	47,XY,+11
Deletion	42~43,X,del(Xq),del(2p),der(3),del(17p),–18,1~2 dmin
	46,XY,del(9)(q22)

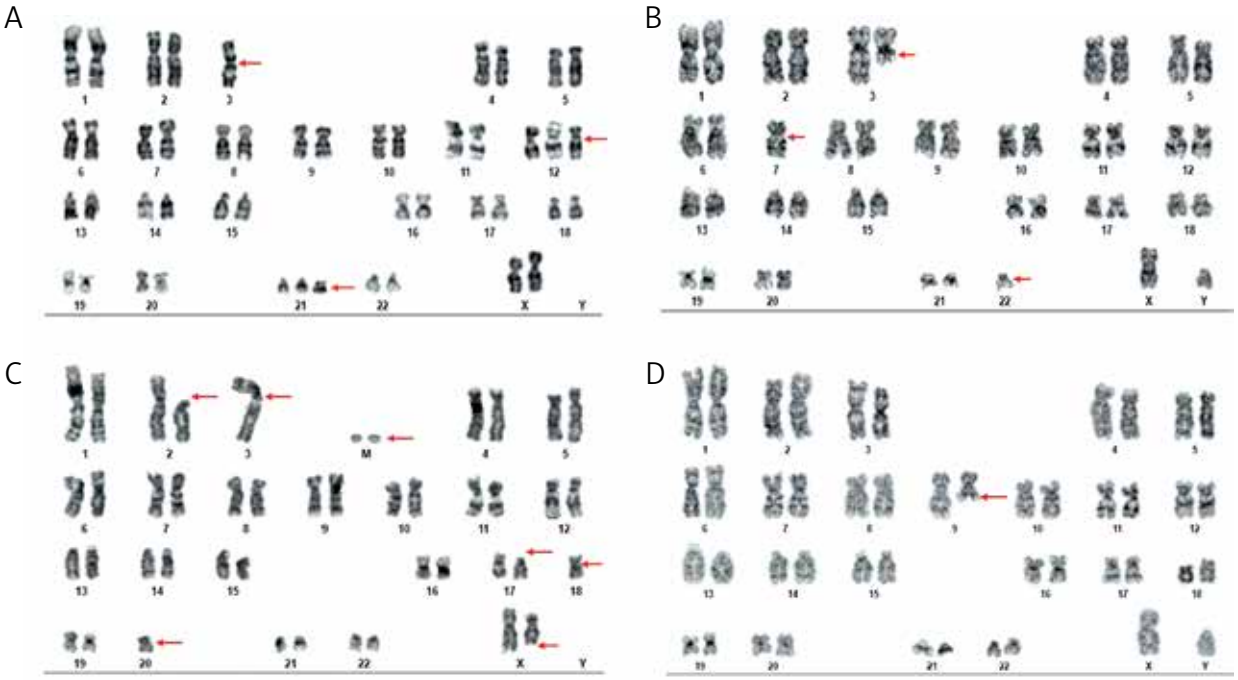

Fig. 2. Hyperdiploidy 47,XX,3,+12,+21 (A), 45,XY,t(3;3)(q22; q29),–7,–22 (B), 42-43,X,del(Xq),del(2p),der(3), del(17p),1~2 dmin (C), 46,XY,del(9)(q22) (D)

of the metaphases for analysis and hence were considered CF cases, which were subjected to FISH to screen the expected chromosomal abnormalities. Abnormal karyotypes constituted structural and numerical abnormalities. Structural abnormalities of translocations were seen in 3 cases and deletions in 2 cases. Numerical abnormality of hypodiploidy was in 6 cases, composite karyotype of 39~50,XX, +1,+2,+3,+9,+12,+20,+mar,–5,–7,–12,–15, –16,–18,–19,–20,X[cp7] in 1 case (Fig. 2), and trisomies and hyperdiploidy were observed in 2 cases separately.

Recurrent translocation of t(15;17)(q24;q21) in (n = 2, 12.5%); monosomy of chromosome 7 was associated as secondary aberration with t(3;3)(q22;q29) in (n = 1, 6.25%); deletions of chromosomes del(9q22) in (n = 1, 6.25%); del(2p); del(17p); del(Xq); 1~2 dmin; and derivative of chromosome 3 was also reported in (n = 1, 6.25%) case. Frequent trisomy of chromosomes 11 and 13 were reported in each case (n = 1, 6.25%); hypodiploidy with random missing of chromosomes in (n = 6, 37.5%), hyperdiploidy in (n = 2, 12.5%), and composite karyotype in (n = 1, 6.25%) were observed. With respect to hypodiploid cases, chromosome 16 was missing in 6 cases, and chromosomes 7, 10, 18, and 20 were missing in 4 cases each. Hyperdiploidy was reported in 2 cases with numerous random gains of chromosomes 12 and 21 (Fig. 3).

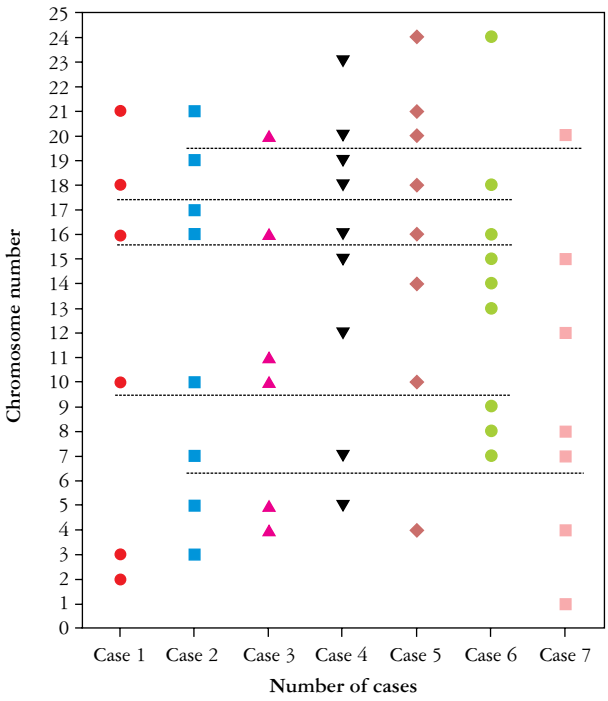

Fig. 3. Representation of hypodiploidy cases associated with all chromosomes

Chromosome number 23 indicates X chromosome.
Chromosome number 24 indicates Y chromosome.

Fluorescence in situ hybridization

Fluorescence in situ hybridization was done on 6 AML cases, 5 ALL cases, and one AL case, comprising 8 CF cases, 2 trisomies, and 2 translocations of specific chromosomal abnormalities. All CF cases showed the normal signal pattern for the probes as mentioned above. Trisomy 11 in one case and Trisomy 13 in another case was confirmed with an MLL (11q23) Break-apart probe and Del(13q) probe, respectively, with additional signal (Fig. 4). Two PML/RARα t(15;17)(q24;q21)

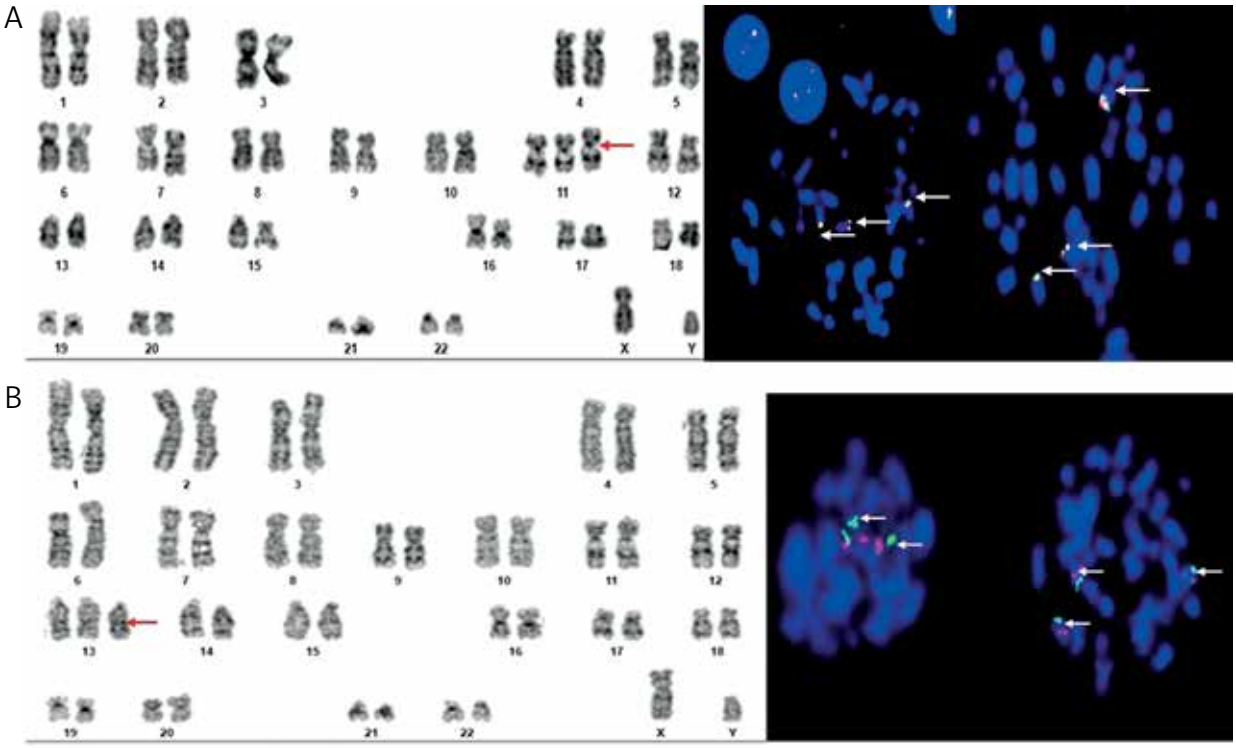

Fig. 4. Trisomy 11, 47,XY,+11 (A), Trisomy 13, 47,XY,+13 (B)

cases were confirmed by 2 yellow fusion signals in addition to the red and green signals of normal chromosomes 15 and 17, respectively (1R, 1G, 2Y).

Discussion

Cytogenetic evaluation in AL is associated with a wide variety of chromosomal abnormalities that directly impact the risk classification, diagnosis, and prognosis of leukaemia diseases. This study shows that AL is more frequently observed in males (69.4%) than in females (30.95%). The proportion of AML in the present study was predominant in the age group > 50 years in 11 cases (50%). In contrast, the study conducted by Ahmad *et al.* showed a predominance in the age group 16–50 years in 120 patients (60%), which might be due to an increased sample number [8]. The incidence of ALL reported in our study was leading in the age group > 16 years in 9 (64.28%) cases and 0–16 years (57.1%). Another study showed that leading in the age group > 16 years was observed in 19 (28.35%) cases and 0–16 years in 48 (71.65%), respectively [9].

The cytogenetic abnormalities obtained from karyotyping indicate a better outcome for critical diagnosis and prognostic information [10]. The success rate of this technique depends clearly on the sample condition, sample processing methods, type of disease, and presence of metaphase spreads [11]. The success rate of these parameters varies from lab to lab, and our success rate was 81.95% (34/42). There were slight variations in the success rate described by Heng *et al.* and Ito *et al.*, which was 90% (185/204) and 87% (112/129), respectively [12, 13]. The bone marrow culture of 19.09% (8/42) cases did not yield any metaphases in the present study due to a low WBC count. Comparatively, by Safaei *et al.* constituting 17.08% (27/168), our success rate was slightly high. Geographic heterogeneity from various locations in AL was published in different studies. The incidence rate of normal karyotypes in our study was 42.85% (18/42) of cases, correlating with previous studies reporting 48.32% (43/89) by Siddaiahgari *et al.* and 32.73% (55/168) by Safaei *et al.* (55/168) as a result of better culture techniques [14, 15].

The present study included paediatric and adult patients with a median age of 40.5 years, which is higher than the 38 years in the study published by Amare *et al.* and lower than the study conducted by Mrozek *et al.* at 58 years [16, 17]. Abnormal Karyotype was observed in 16 (39.09%) cases, which constituted hypodiploid, hyperdiploid, composite karyotype, translocations, trisomies, and deletions. Hypodiploidy and hyperdiploidy were the 2 most prominent cytogenetic abnormalities present in our study, with 7 cases associated with random missing chromosomes. Concerning hypodiploid cases, chromosome 16 was missing in 6 cases, while chromosomes 7, 10, 18, and 20 were missing in 4 cases each. Hence, these chromosomes were frequently missing in this group of patients, which were rarely reported. Hyperdiploidy was reported in 2 cases, with numerous gains in random chromosomes. Hyperdiploidy

Table IV. Frequency of various cytogenetic studies reported in acute leukaemia

Author	Present study	Gandhia, Pankaj [10, 34]	Ahmad [9]	Safaei [14]	Siddaiahgari [15]	Bao [32]	Preiss [33]
Year	2022	2018	2008	2013	2015	2006	2004
Country	India	India	India	Iran	India	China	Denmark
No. of subjects	42	35	75	168	103	174	337
Median age	41	–	–	–	4	50	67
No. abnormality (%)	39.09	68.57	43.3	73.07	–	38	47
Males/females	29/13	25/10	48/27	114/54	73/30	–	–
AML/ALL	Both	ALL	ALL	ALL	ALL	AML	AML
Paediatric/adult	Both	Both	Paediatric	Both	Paediatric	Adult	Adult
Hypodiploidy	6	11.11	10.34	6	2.25	–	–
Hyperdiploidy	2	11.43	20.68	45	10.11	–	–
Translocation	3	83.3	17.2	1	12.35	17	6.6
Trisomy	2	–	3.4	16	1.12	13.8	8.9
Deletions	2	8.3	17.24	5	6.74	18	25.7
Others	1	37.5	17.32	13	17.99	6.6	4.2

ALL – acute lymphocytic leukaemia, AML – acute myeloid leukaemia

also includes chromosomal gains significantly with trisomies, tetrasomies, monosomies, and more than 2 chromosomes. The majority of hyperdiploidy cases are seen with +X,+4,+6,+10,+14,+17,+18, or +21 [18], whereas our study reported the gain of chromosomes +12 and +21. Two hyperdiploidy cases were present in adult patients in AML and ALL. Hypodiploidy and hyperdiploidy were frequently reported in Indian study groups. It is associated with low WBC counts with a favourable prognosis and overall survival rates > 90% based on current treatment protocols. The present study was compared with various studies reported and mentioned in Table IV, which includes the frequency of various cytogenetic abnormalities.

The t(15;17) occurs in 5–10% in AML, which is a reciprocal balanced translocation involving the fusion of PML located on 15q22 and RARα chain genes on 17q12-21 chromosomes [19]. The t(15;17) is regularly screened as the sole abnormality, as reported in our study as well, but in some complex cases it is associated with other common chromosomal aberrations such as +8, del(7q), del(9q), and der(17)(q10) [19]. The t(15;17) reported in our study was present in one paediatric and one adult female AML case with 5 and 53 years, respectively. The t(3;3)(q21;q26.2) with monosomy of chromosome 7 is a rare cytogenetic abnormality with poor prognosis, which was reported in our study in one adult case with 50 years. The t(3;3)(q21;q26.2) reported by Sitges *et al.* was a sole abnormality without associating with any other secondary cytogenetic abnormalities. Guiding to the juxtaposition of the ecotropic viral integration site-1 (EVI1) gene with the ribophorin 1(RPN1) gene as homologous reciprocal translocation resulting in transcriptional activation of the EVI1 gene playing a critical role in the pathogenesis of the myeloid neoplasm's driving cellular proliferation [20, 21]. Monosomy 7 associated with t(3;3) reported in our study has an adverse effect in AML, commonly seen with other cytogenetic abnormalities like complex karyotype, monosomy 5/ del 5, del7q, and inversion of chromosome 3 [22].

Trisomies of +4,+8,+11,+13, and +21 are the most frequently reported in AL study groups. Our study reported sole trisomies 11 and 13 in adult male patients of 2 different cases, which are the most frequent numerical chromosomal abnormalities seen in AL, with a 3% incidence rate in elderly patients [23]. Trisomies occur as both sole as well as secondary chromosomal abnormalities in AL. The studies conducted by Ahmad *et al.* reported trisomies +11 and +13 in female paediatric patients. According to the 2017 European Leukaemia Net classification, patients with any sole trisomy are classified under intermediate risk, leading to no mutations in the coexisting genes, so they are reclassified into favourable or adverse risk groups [24]. Trisomies are associated with mutational landscape with many genes like MLL-PTD, DNMT3A, U2AF1, FLT3-ITD, and IDH2, which are known through high-end techniques of next-generation sequencing to understand the pathogenesis of AL [25].

Deletions are one of the recurrent chromosomal abnormalities generally occurring as sole, with an incidence rate of 2% [26]. Del 17p results in the inac-

tivation of major tumour suppressor genes, resulting in poor outcome of the AML when subjected to chemotherapy [27]. Del 9q was reported in our study as the sole abnormality, a characteristic feature correlating with the study conducted by Peniket *et al.* in which Del 9q was reported as sole and associated with t(8;21) [28]. Del 9q can be considered as an intermediate prognostic factor in leukaemia, which is consistent with the report by Dohner *et al.* Sex chromosomal abnormalities are not frequently observed in haematological malignancies. Cytogenetic abnormalities affecting chromosome X are mostly included with numerical ones, with either gain or loss of the whole chromosome, while the loss of Y chromosomes is frequently seen in myelodysplastic syndrome [29]. Deletion of the long arm of chromosome X is rare in myeloid malignancies, and this was also obtained from our study, which also correlates with the study conducted by Wong *et al.*, in which del(X)(q13–q28) regions the loss of genetic materials were clustered. Double minutes (d min) of 1~2 have been reported in one case in our study, which is common in solid tumours and rare in haematological malignancies. The most common gene amplification in AL frequently [30] includes C-MYC and MLL genes associated with the formation of d min. Variations in chromosomal abnormalities from one cell to another cell resulting in composite karyotype we reported in one case of our study 39~50, XX,+1,+2,+3,+9,+12,+20,+mar,–5,–7,–12, –15,–16,–18,–19,–20,X[cp7]. Genetic heterogeneity is mostly seen in neoplastic syndromes and not with constitutional disorders; regardless, a patient has undergone some radiation or chemotherapeutic treatment when a chromosome analysis is performed in haematological malignancies [31].

Conclusions

Conventional cytogenetics plays a vital role in the clinical diagnosis, prognosis, and therapeutic approach of acute leukaemia. The molecular findings help in ruling out the genetically mutated genes in the pathophysiology of acute leukaemia. This study was conducted to find the prevalence of recurrent cytogenetic and molecular alterations in acute leukaemia. The chromosomal abnormalities obtained from our research had different incidence rates than other research groups, which might be due to environmental factors, high exposure to carcinogenic agents, and differences in ethnicity and geographical conditions. Hypodiploidy was associated more than hyperdiploidy in this study, which signifies the loss of chromosomes observed randomly in the South Canara region, where the present study was conducted. We also screened out rare cytogenetic translocations, composite karyotypes, and double minutes. Despite other advanced molecular techniques in diagnosing the neoplasm, conventional cytogenetics remains the baseline in the diagnostics of malignancies. Further molecular studies with gene mutational and next-generation sequencing would help better understand the in-depth mechanism of acute leukaemia.

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References

1. Gomez-Almaguer D, Marcos Ramirez ER, Montano-Figueroa EH, et al. Acute leukemia characteristics are different around the world: the Mexican perspective. Clin Lymphoma Myeloma Leuk 2017; 17: 46-51.
2. Daver N, Schlenk RF, Russell NH, Levis MJ. Targeting FLT3 mutations in AML: a review of current knowledge and evidence. Leukemia 2019; 33: 299-312.
3. Pui CH, Relling MV, Downing JR. Acute lymphoblastic leukemia. N En J Med 2004; 350: 1535-1548.
4. Mrozek K, Heerema NA, Bloomfield CD. Cytogenetics in acute leukemia. Blood Rev 2004; 18: 115-136.
5. Nachman JB, Heerema NA, Sather H, et al. Outcome of treatment in children with hypodiploid acute lymphoblastic leukemia. Blood 2007; 110: 1112-1115.
6. Arumugam M, Shetty DP, Kadandale JS, Kumari SN. Y chromosome microdeletion and cytogenetic findings in male infertility: a cross-sectional descriptive study. Int J Rep Bio Med 2021; 19: 147.
7. Shaffer LG, McGowan-Jordan J, Schmid M. ISCN 2013: an international system for human cytogenetic nomenclature. Switzerland: Karger Medical and Scientific Publishers, Basel 2013.
8. Ahmad F, Dalvi R, Das BR, Mandava S. Specific chromosomal aberrations in de novo acute myeloid leukemia: a comparative analysis of results with a report of three novel chromosomal rearrangements t(7;14)(q35;q13), t(8;18)(p11.2;q12), t(13;15) in Indian population. Cancer Detect Prev 2008; 32: 168-177.
9. Ahmad F, Dalvi R, Chavan D, Das BR, Mandava S. Cytogenetic profile of acute lymphocytic leukemia patients: report of a novel translocation t(4;13)(q21·3;q35) from an Indian population. Hematology 2008; 13: 28-33.
10. Gadhia P, Parekh N, Chavda P, Vaniawala SN. Cytogenetics findings of patients with acute lymphoblastic leukemia in West Indian Region. Int J Adv Res 2014; 6: 563-570.
11. Mazloumi SH, Kumari P, Madhumathi DS, Appaji L. Rare and recurrent chromosomal abnormalities and their clinical relevance in pediatric acute leukemia of south Indian population. Indian J Med Paed Oncol 2012; 33: 166-169.
12. Heng JL, Chen YC, Quah TC, Liu TC, Yeoh AE. Dedicated cytogenetics factor is critical for improving karyotyping results for childhood leukaemias-experience in the National University Hospital, Singapore. Ann Acad Med Singapore. 2010; 39:102-106.
13. Ito C, Kumagai MA, Manabe A, et. al. Hyperdiploid acute lymphoblastic leukemia with 51 to 65 chromosomes: a distinct biological entity with a marked propensity to undergo apoptosis. Blood 1999; 93: 315-320.

14. Safaei A, Shahryari J, Farzaneh MR, Tabibi N, Hosseini M. Cytogenetic findings of patients with acute lymphoblastic leukemia in Fars Province. Iran J Med Sci 2013; 38: 301-307.
15. Siddaiahgari S, Awaghad M, Latha M. Clinical, immunophenotype and cytogenetic profile of acute lymphoblastic leukemia in children at tertiary health care centre in India. Muller J Med Sci Res 2015; 6: 112.
16. Amare PK, Jain H, Kabre S, et. al. Cytogenetic profile in 7209 Indian patients with de novo acute leukemia: a single centre study from India. J Cancer Ther 2016; 7: 530-544.
17. Mrozek K, Marcucci G, Nicolet D, et. al. Prognostic significance of the European LeukemiaNet standardized system for reporting cytogenetic and molecular alterations in adults with acute myeloid leukemia. J Clin Oncol 2012; 30: 4515.
18. Paulsson K, Johansson B. High hyperdiploid childhood acute lymphoblastic leukemia. Genes Chromosomes Cancer 2009; 48: 637-660.
19. Wan TS, So CC, Hui KC, Yip SF, Ma ES, Chan LC. Diagnostic utility of dual fusion PML/RARα translocation DNA probe (D-FISH) in acute promyelocytic leukemia. Oncol Rep 2007; 17: 799-805.
20. Wang ZY, Chen Z. Acute promyelocytic leukemia: from highly fatal to highly curable. Blood 2008; 111: 2505-2515.
21. Sitges M, Boluda B, Garrido A, et al. Acute myeloid leukemia with inv(3)(q21.3q26.2)/t(3;3)(q21.3;q26.2): study of 61 patients treated with intensive protocols. Eur J Haematol 2020; 105: 138-147.
22. Poire X, Labopin M, Polge E, et. al. The impact of concomitant cytogenetic abnormalities on acute myeloid leukemia with monosomy 7 or deletion 7q after HLA-matched allogeneic stem cell transplantation. Am J Hematol 2020; 95: 282-294.
23. Bhatnagar B, Eisfeld AK, Kohlschmidt J, et al. Clinical and molecular characterization of patients with acute myeloid leukemia and sole trisomies of chromosomes 4, 8, 11, 13 or 21. Leukemia 2020; 34: 358-368.
24. Dohner H, Estey E, Grimwade D, et al. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. Blood 2017; 129: 424-447.
25. Holmfeldt L, Wei L, Diaz-Flores E, et. al. The genomic landscape of hypodiploid acute lymphoblastic leukemia. Nat Genet 2013; 45: 242-252.
26. Yang JJ, Park TS, Wan TS. Recurrent cytogenetic abnormalities in acute myeloid leukemia. Methods Mol Biol 2017; 1541: 223-245.
27. Poire X, Labopin M, Maertens J, et al. Allogeneic stem cell transplantation in adult patients with acute myeloid leukaemia and 17p abnormalities in first complete remission: a study from the Acute Leukemia Working Party (ALWP) of the European Society for Blood and Marrow Transplantation (EBMT). J Hematol Oncol 2017; 10: 1-10.
28. Peniket A, Wainscoat J, Side L, et al. Del (9q) AML: clinical and cytological characteristics and prognostic implications. Br J Haematol 2005; 129: 210-220.
29. Wong KF, Siu LL, So CC. Deletion of Xq23 is a recurrent karyotypic abnormality in acute myeloid leukemia. Cancer Gen Cytogenet 2000; 122: 33-36.
30. Wong KF, Siu LL, Wong WS. Double minutes and MYC amplification: A combined May-GrünwaldGiemsa and fluorescence in situ hybridization study. Am J Clin Pathol 2014; 141: 280-284.
31. Zneimer SM. Cytogenetic abnormalities: chromosomal, FISH, and microarray-based clinical reporting and interpretation of result. John Wiley & Sons, Hoboken 2014, 1-8.
32. Bao L, Wang X, Ryder J, et al. Prospective study of 174 de novo acute myelogenousleukaemias according to the WHO classification: subtypes, cytogenetic features and FLT3 mutations. Eur J Haematol 2006; 77: 35-45.
33. Preiss BS, Kerndrup GB, Schmidt KG, et al. Cytogenetic findings in adult de novo acute myeloid leukaemia. A population-based study of 303/337 patients. Br J Haematol 2003; 123: 219-234.
34. Pankaj KG. Role of cytogenetic evaluation in diagnosis of acute myeloid leukaemia. Am J Biomed Life Sci 2016; 4: 98-102.

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