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Comprehensive Genetic Analysis by Integration of Conventional Karyotyping and Interphase FISH Helps Refinement of Biological Subclasses with Clinical Impact in Chronic Lymphocytic Leukemia

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Abstract

Background: Various genetic technologies have been employed in the identification of genomic complexity and refinement of prognostic classification of clinically heterogeneous disease of chronic lymphocytic leukemia (CLL). Objective: The present study of interphase cytogenetics and conventional karyotyping was undertaken to perform comprehensive analysis of CLL genetics with an approach to refine early prognostication of disease. Material & Methods: Retrospective analysis by fluorescence *in situ* hybridization (FISH) was carried out on total 671 patients of CLL at diagnosis between 2008 and 2015. Conventional cytogenetics studies were performed in 50 of 671 patients using CPG Oligonucleotide + IL-2 and TPA (12-O-Tetradecanyl Phorbol 13-acetate) for stimulation of lymphocytes cultures. Results: Interphase cytogenetics could detect recurrent abnormalities such as del(13q14), +12, del(17p13), del(11q22), del(6q23) in 71% of cases. The incidence of del(13q) was higher in Rai stage 0, I, II (p = 0.0005); whereas patients with ≥2 aberrations were more common in advance stage III, IV (p = 0.001). Frequency of IgH translocation was 7%. Morphology and immunophenotypic analysis revealed atypical CLL with higher frequency of t(14;19) than t(14;18). Conventional karyotype could detect abnormal karyotype in 97% of cases which displayed targeted FISH abnormalities along with additional non-targeted chromosomal abnormalities. Patients with negative FISH markers showed clonal non-recurrent numerical and

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structural changes. The complex karyotype was identified in 24% cases which included targeted FISH aberrations as well as non-targeted numerical and structural abnormalities like deletions, and unbalanced translocations. A significant association was observed between complex karyotype and coexistence of ≥2 FISH markers (p = 0.009) and del(11q22) &/or del(17p) (p = 0.03). Conclusion: Our data of interphase FISH with integration of conventional karyotyping revealed genomic complexity that helped identification of biological subclasses with clinical impact at diagnosis. Further, these cytogenetic subclasses along with molecular markers are likely to evolve more refined prognostic groups, which will help design risk-adapted therapies in B-CLL.

Keywords

CLL, FISH, Complex Karyotype, Biological Subclasses, Prognostic Groups

1. Introduction

Chronic lymphocytic leukemia (CLL) is a clinically heterogeneous disease with variable response to chemotherapy, survival period from less than one year to more than 15 years [1]-[4]. Rai and Binet staging has limitations with respect to early prediction of chemotherapy response, progression or future course of disease [3]-[6]. Over a period of two decades, various genetic technologies like Fluorescence *In-situ* Hybridization (FISH), conventional karyotyping, genomic microarrays, gene sequencing have played a significant role in the refinement of genomic complexity [7]-[19]. Donher *et al.* [20] who established interphase cytogenetics in B-CLL, is still a gold standard, simple laboratory technique, able to detect recurrent chromosomal abnormalities, del(13q), trisomy 12, del(11q), del(17p), del(6q) with distinct prognosis in 70% - 80% of CLL patients [9]-[12] [17] [21].

Although gold standard, targeted nature of interphase cytogenetics limits its contribution in the assessment of comprehensive genomic assessment, the detection of additional clonal abnormalities apart from recurrent aberrations by conventional karyotyping helps identification of different genetic subclasses with distinct prognostic classification [8] [9] [14] [22]-[24].

Recently, complex karyotype with *IgVH* mutation has been found to be a high risk group in CLL [7]-[9] [14] [25] [26]. Attention was also focused to 14q32 translocation involving *IgH* gene [27]-[29]. Cavazzini *et al.* (2011) [29] found clonal evolution in complex karyotypes in cases with *IgH* translocation and these cases were associated with chemorefractory to standard/targeted regimens.

The present study was undertaken 1) to evaluate the frequency of recurrent interphase FISH abnormalities which include del(13q14), +12, del(11q22), del(17p13), del(6q23) and correlate these markers with clinico-pathological parameters, 2) to evaluate frequency and characterization of *IgH* translocations and their association with typical & atypical CLL, 3) to investigate comprehensive analysis of genetic picture by conventional karyotyping, & 4) to correlate complexity and/or non-complexity of karyotypic picture with FISH results & clinical variables.

2. Material & Methods

A total of 671 CLL patients (512 Males & 159 Females, Age Range: 24 - 92 years, Median Age: 58 years, M:F Ratio: 3.2) diagnosed by standard morphology and immunophenotypic criteria between May, 2008 and Dec, 2015 at the Department of Medical Oncology, Tata Memorial Hospital, were included in the present study. The diagnosis of atypical CLL included morphology (nuclear indentation) and immunophenotype (CD 22+ve, CD 23 dim/−ve, surface Ig weak and FAM7 +ve). FISH and conventional karyotyping studies were performed at diagnosis before the decision of treatment initiation. Those patients who needed treatment were treated with standard chemotherapy including Fludarabin, Chlorambucil and Cyclophosphamide.

FISH was performed on interphase cells from bone marrow aspirate and/or peripheral blood with panel of probes that includes LSI D13S319(13q14.3)/LSI 13q34, CEP 12, LSI(17p13.1)/D17Z1, LSI *ATM*(11q22)/D11Z1, LSI *MYB*(6q23)/D6Z1, LSI *IgH* break apart, LSI dual fusion *IgH*/*CCND*1, *IgH*/*BCL*2, *IgH*/*BCL*3 and *C-MYC* break apart probe (Abbott Molecular, Delkenheim, Germany and Kreatech Diagnostics, The Netherland). FISH protocol followed as described previously [17] [20]. Minimum 200 cells were scored in each spe-

cimen. The cut off threshold for trisomy and dual fusion probes was 2%. The cut off threshold for LSI 13q14 deletion, *ATM* deletion, and 6q deletion was 5%. The baseline value for *TP*53 deletion and break apart probe for *IgH* & *C-MYC* was 7%. Patients with ≥2 FISH markers were grouped under coexistence of ≥2 aberrations. *IgH* translocation studies were carried out in a cohort of 557 out of 671 patients (Age Range: 37 - 88 Years).

Conventional karyotyping was performed in 50 patients from a cohort of 671 patients. The target materials were bone marrow aspirate and/or peripheral blood which were cultured in HAM F10 with 10% fetal calf serum along with immunostimulatory CPG oligonucleotide DSP 30 (2 μM) (TIB MOL BIOL, Berlin, Germany) in combination with Interleukin-2 (IL-2) (200 units/ml) (Roche, Sydney, Australia). Simultaneously, cultures were also stimulated with TPA (12-O-tetradecanoyl phorbol 13-acetate) (50 ng/ml) [7]. After 72 hrs of stimulation, cultures were terminated and chromosome preparation was done as per standard protocol. The correlation of FISH markers with clinical variables and with conventional karyotyping findings was evaluated by Pearson's chi-square test (SPSS version 20).

3. Results

3.1. FISH Findings at Diagnosis

Table 1 summarizes frequency of FISH markers including del(13q14)/-13, +12, del(11q22), del(17p13), del(6q23) in a cohort of 671 patients (**Figure 1**). The frequency of FISH recurrent markers was 71% (477/671).

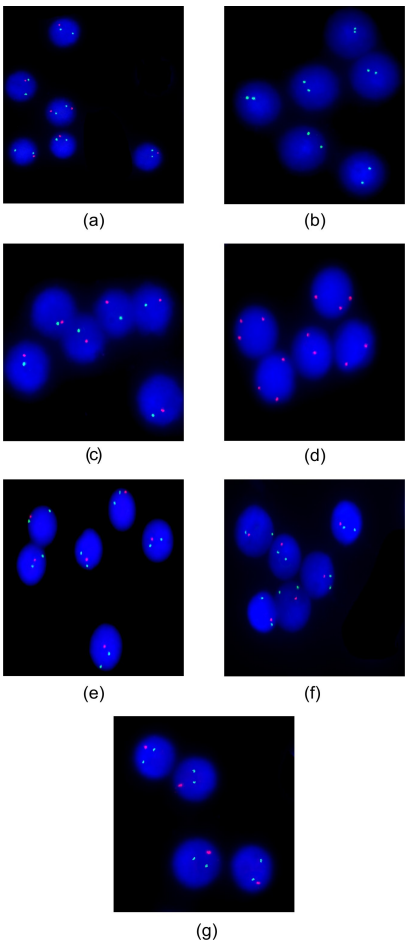

Figure 1. (a) Interphase FISH with LSI 13S319(13q13.4) and LSI 13q34 (control) shows heterozygous, monoallelic 13q14 deletion (1R2G); (b) Biallelic, homozygous 13q14 deletion (0R2G); (c) Monosomy 13(1R1G); (d) CEP 12 probe shows trisomy 12(3R); (e) LSI *ATM*(11q22) and D11Z1 (control) shows heterozygous 11q22 deletion (1R2G); (f) LSI *TP*53 (17p13.1) and D17Z1 (control) probes show heterozygous *TP*53 deletion (1R2G); (g) LSI *MYB*(6q23) and D6Z1 (control) probes show heterozygous 6q23 deletion (1R2G).

A 13q biallelic deletion was detected in 30/288 (10%) cases (**Figure 1(b)**). There was no association of monoallelic/biallelic deletion with stage of the disease (Mono 90% & Bi 10% in 0, I, II vs 87% & 13% in III, IV (p = 0.52). The co-existence of ≥2 aberrations was detected in 139/477(29%) of FISH positive group. The clone size of aberrant FISH markers was 5% - 95%. Among the FISH positive group, Abn(13q) occurred as a most frequent sole abnormality: 38% (181/477) followed by trisomy 12: 17% (83/477), del(11q), del(17p): 6% (29-30/477) each and del(6q): 8% (15/477). In a group of ≥2 aberrations, Abn(13q) was most common, 22% (107/477), followed by Abn(17p), 15% (71/477), del(11q), 12% (58/477), trisomy 12, 9% (45/477) and del(6q), 7% (36/477). In a cohort of 477 cases of positive FISH markers, Rai staging was available in 432 cases, of which 183 and 249 cases were in stage 0, I, II and III, IV respectively. Among all FISH positive markers, del(13q)/-13 was associated with lower age group (p = 0.02).The correlation of Rai staging system with FISH markers revealed association of Abn(13) with lower stage group (0, I, II) (p = 0.0005); on the other hand ≥2 aberration positive FISH group revealed association with advanced stage (III & IV) (p = 0.001) (**Table 2**).

The frequency of *IgH* translocations in a cohort of 557 cases was 7% (42/557). The translocations were characterized as t(14;18)(q32;q21)(41%:17/42), t(14;19)(q32;q13)(24%:10/42), t(8;14)(q32;q24)(2%:1/42) and variant *IgH* translocations (33%:14/42) (**Figure 2**). In 32 out of 42 cases, morphology details and immunophenotypic analysis revealed atypical CLL in 20 cases, in which frequency of t(14;19) was higher in comparison with t(14;18) (**Table 3**, **Figure 3**). The CD38 was more common in t(14;19)(5/7) and t(14;18)(7/13) positive cases.

Table 1. Frequency of FISH targeted markers by FISH in CLL in present study (n = 671) and incidence in reported literature.

Cytogenetic Marker	No. of Patients (%)	Incidence in Reported Literature
del(13q)/-13	288(43)	40% - 60%
Trisomy 12	127(19%)	20% - 40%
del(11q22)	87(13%)	10% - 25%
del(17p13)/-17	101(15%)	4% - 15%
del(6q23)	51(8%)	5% - 9%

Table 2. Correlation of FISH genetic groups with age and stage of the disease (n = 432).

FISH Genetic Groups	Age (years)		p value	Rai Stage		P value
	<58	>58		0, I, II	III, IV	
	Positive (%)	Negative (%)		Positive	Negative	
del(13q)/-13	67(42)	168(53)	0.028	103(71)	146(51)	0.0005
	92(58)	150(43)		43(29)	140(49)	
Trisomy 12	31(43)	204(50)	0.253	36(59)	213(57)	0.814
	41(57)	201(50)		25(41)	158(43)	
del(11q22)	14(61)	221(49)	0.155	10(48)	239(58)	0.341
	9(39)	223(51)		11(52)	172(42)	
del(17p13)/-17	18(62)	217(48)	0.155	15(60)	234(58)	0.806
	11(38)	231(52)		10(40)	173(42)	
del(6q23)	8(62)	227(49)	0.370	6(50)	243(58)	0.587
	5(38)	237(51)		6(50)	177(42)	
≥2 aberrations	65(50)	170(49)	0.766	54(45)	195(63)	0.001
	64(50)	178(51)		67(55)	116(37)	

Table 3. Frequency of t(14;18), t(14;19), t(8;14) and variant *IgH* translocations in Atypical and Typical CLL (n = 32).

IgH Translocation	Atypical CLL	Typical CLL
t(14;18)	7(54%)	6(46%)
t(14;19)	5(71%)	2(29%)
t(8;14)	1(100%)	0
Variant *IgH* translocations	7(63%)	4(37%)

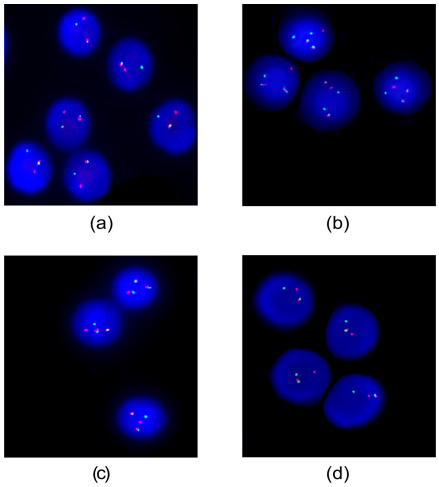

Figure 2. (a) Interphase FISH with LSI *IgH* dual colour break apart probe shows *IgH* translocation (1R1G1Y); (b) Dual fusion *IgH/BCL2* translocation probe shows standard *IgH/BCL2* (1R2G2Y); (c) Dual fusion *IgH/BCL3* translocation probe shows IgH/BCL3 (1R1G2Y); (d) LSI *C-MYC* dual colour break apart probe shows *C-MYC* translocation (1R1G1Y).

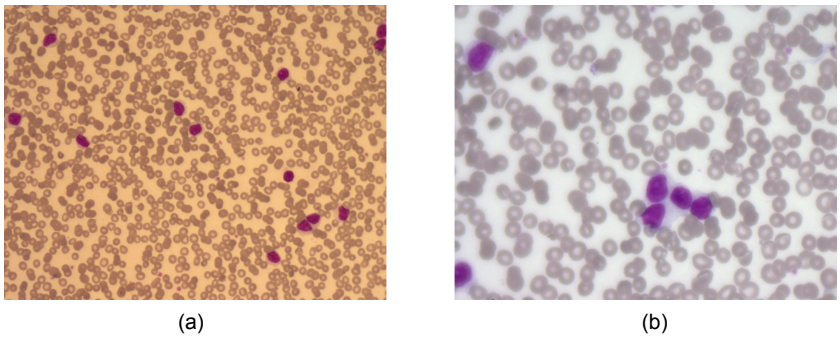

Figure 3. Bone marrow morphology shows heterogeneous mixture of small and medium sized lymphocytes with indented nuclei.

IgH translocations were most frequently accompanied by trisomy 12(53%:22/42) followed by del(13q) (28%: 12/42) (**Table 4**). Clone size of *IgH* translocation, trisomy 12 and del(13q) revealed that *IgH* translocation was a primary event.

3.2. Conventional Karyotyping (CK) Findings and Comparison of FISH and Conventional Karyotyping (Table 5)

Of total 50 specimens, successful cultures were obtained in 40(80%) specimens. Both CPG-oligonucleotide DSP 30 +IL-2 and TPA stimulation showed almost equal proliferative index. A minimum of 10 - 20 metaphase cells were karyotyped in each specimen. Of total 40, 31 preparations with 200 - 300 G-band resolution were karyo-

typed and of the total 30/31 specimens (97%) displayed abnormal karyotype (**Figure 4**, **Figure 5**).

Interestingly, 2/11 cases with no targeted abnormalities by FISH displayed clonal abnormalities del(13)(q14q22) (**Figure 4(b)**) and trisomy 12 by CK; whereas 7/11 cases with negative FISH markers showed deletions affecting chromosomal loci 8p11, 9p11, 11q22-23, 12p13, 14q11.2-q24, 15q11.2-15 (**Figure 4(d)**), 15q22.3-24, 18p11, balanced translocations t(1;14)(q42;q32), t(14;18)(q32;q21) and unbalanced 14q32 translocation. Overall 29% (9/31) cases with negative FISH markers revealed either targeted FISH markers and/or other non-recurrent chromosomal abnormalities. The remaining 21 out of 31 cases with targeted abnormalities by FISH panel of probes revealed the same abnormalities in clonal form in respective karyotypes (**Table 5**). Two cases with targeted FISH aberrations, del(17p) & del(11q) disclosed additional targeted /non-targeted abnormalities such as del(13q14q22) and del(6q22). Of 21 cases with targeted FISH abnormalities, 15 cases displayed additional numerical abnormalities, gains and losses of various chromosomes *viz*, +6, −8, +8, +11, −14, −18, +19, +20, +21, +22 and structural abnormalities such as del(1)(q21), del(1)(q11), del(1)(q25), i(1p), del(4p15), del(7q32), del(13)(q12q14), del(13)(q14q22), del(16q22), del(15q24), del(18p11), unbalanced translocations, t(14q32), t(7p22), t(11q21), t(12p13), t(14p11), t(10q24), t(15q25) (**Figure 4(c)**), and balanced translocations t(13;14)(q22;q32) (**Figure 4(a)**). Overall 71% (22/31) cases had heterogeneous chromosomal abnormalities.

Table 4. Association of FISH markers [+12, del(13q/-13), del(11q22)] with *IgH* translocations.

IgH Translocation	Trisomy 12	del(13q)/-13	del(11q22)	Sole Aberration
t(14;18)	46%	31%	-	23%
t(14;19)	71%	28%	-	-
t(8;14)	-	-	-	100%
Variant *IgH* Translocations	45%	9%	9%	36%

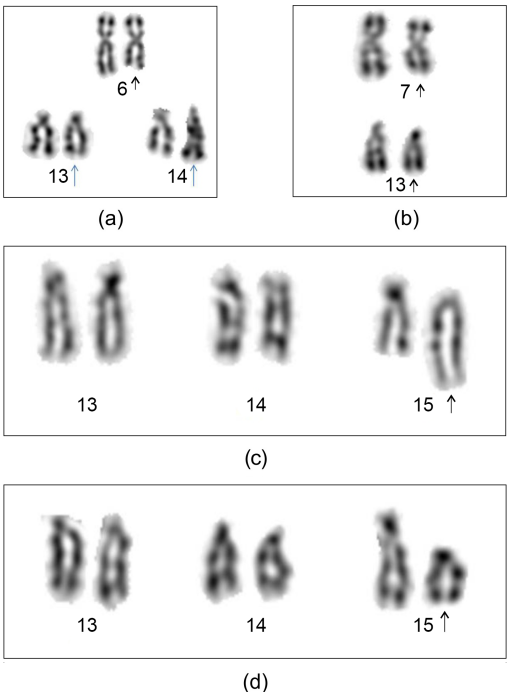

Figure 4. (a) Partial karyotype of a case with del(13q) by FISH shows del(6)(q23) and t(13;14)(q22;q32); (b) Partial karyotype of a case with del(13q) by FISH shows del(13)(q14q22), del(7)(q32); (c) Partial karyotype of a case with del(13q) by FISH shows t(?;15)(?;q25) as an additional abnormality; (d) Partial karyotype of a case with negative FISH shows del(15)(q11.2q15).

Table 5. Karyotyping and FISH markers details in the present study (n = 31).

Sr.No	Age	Sex	Rai Stage	Karyotype	FISH markers: del(13q)/-13, Abn(17p)/-17, del(11q), +12, del(6q)
1	66	M	II	46, XX, del(18)(p11) [2]/46, XX, del(18)(p11) [3], del(11)(q23) [2], inv(7)(p12q22) [2] [cp7]/47, +mar [2]/46, XX [2]	Negative
2	68	M	I	47, XY, +12 [10]/46, XY [2]	nuc ish(CEP12X3)
3	70	M	III	46, XY, del(17)(p13) [5]/46 - 47, XY, del(17)(p13) [2], +21[cp2]/87 - 89, XXY, +X, +1, +2, +3----del(17)(p13) [5]	nuc ish(D17Z1X4-5, *TP*53X2)
4	45	M	I	47, XY, del(7)(q32), +12, del(13)(q14.1q14.3) [2]/47, XY, del(1)(q21) [2], del(7)(q32) [3], del(8)(p11) [2], del(13)(q14q22) [3], t(?;14)(?;q32) [3] [cp7]	nuc ish(CEP12X3) (D13S319X1, 13q34X2)
5	66	F	I	46, XX, del(13)(q14q22) [3]/45, XX, −21 [3]/46, XX [14]	Negative
6	62	M	I	46, XY, t(1;14)(q42;q32) [2]/46, XY [10]	Negative
7	68	M	IV	47, XY, del(19)(p11), +21 [2], +mar [2] [cp3]/46, XY [5]	Negative
8	56	M	0	47, XY, t(?;7)(?;p15), +12 [2]/47, XY, +12, del(13)(q12q14) [2]/49, XY, +12, +19, +mar[2]/48, XY, +6, +8, −10, +12, −18, +mar [3]/46 - 48, XY, +12[2], del(13)(q12q14) [2], +mar [cp8]	nuc ish(CEP12X3)
9	52	M	I	45, XY, −8, del(13)(q14q22), i(17q), −18 [3]/45, XY, −17 [6]/46, XY [2]	nuc ish(D17Z1X2, *TP53*X1), (D17Z1X1, *TP53*X1)
10	70	M	III	46, XY, del(6)(q22), del(11)(q22), del(15)(q24), −18 [3]/46, XY, del(6q22) [3], −8 [3], del(18)(p11) [2] [cp5]/46, XY [2]	nuc ish(D11Z1X2, *ATM*X1)
11	50	F	II	47, XY, +12 [11]/47, XY, +12, del(13)(q14) [5]/ 46 - 48, XY, +12 [2] [cp2]	nuc ish(CEP12X3) (D13S319X1, 13q34X2)
12	64	M	III	47, XY, +1, i(1p), −2, +12 [3]/45, XY, +1, i(1p), −2, +12, −18, +21[3]/47, XY, +1 [2], +12 [2] [cp3]	nuc ish(D11Z1X2, *ATM*X1)
13	71	F	III	46, XX, del(13)(q14) [2]/46, XX, −13[2]/46, XX, −7, −13 [6]/46, XX [2]	nuc ish(D13S319X1, 13q34X2), (D13S319X1, 13q34X1)
14	67	M	0	46, XY, del(13)(q14.1q14.3) [3]/46 - 47, XY, del(13q) [5], t(?;15)(?;q25) [2] [cp5]/46, XY [5]	nuc ish(D13S319X1, 13q34X2)
15	42	M	II	46, XY, t(13;14)(q22;q32) [4]/46, XY, del(4)(q31) [2], del(6)(q23) [2], del(11)(q22-23) [2], del(12)(q24) [2], t(13;14) [2] [cp7]/46, XY [2]	nuc ish(D11Z1X2, *ATM*X1)
16	58	M	IV	47, XY, del(15)(q11.2q15) [5]/47, XY, +8 [2]/47, XY, +mar [3]/46, XY [4]	Negative
17	66	M	0	47, XY, +9, del(12)(p13), del(15)(q15q22) [2]/45 - 46, XY, del(12)(p13) [4], del(15q) [2] [cp4]/46, XY [9]	Negative
18	57	M	I	45 - 46, XY, +9 [2], del(11)(q22) [4], t(?;11)(?;q21) [3], −20 [3] [cp9]/46, XY [4]	nuc ish(D11Z1X2, *ATM*X1)
19	69	M	0	46, XY, +12 [2]/46 - 47, XY, +12 [7]/46, XY [6]	nuc ish(D11Z1X2, *ATM*X1)
20	64	M	0	45, XY, +11, del(11)(q22), −17, −18 [3]/44 - 45, XY, del(6)(q15q21) [2], del(7)(q32) [3], del(11)(q22) [4], +11 [3], t(?;14)(?;p11) [2], −15 [3], −17 [6], +21 [2], +22 [2] [cp7]	nuc ish(D11Z1X2, *ATM*X1), (D17Z1X1, *TP*53X1)

Continued

21	60	M	I	47, XY, +12 [5]/48, XY, +12, +21 [3]/47 - 49, XY, del(1)(q25) [3], +12 [3], +21 [3] [cp4]/46, XY [3]	nuc ish(CEP12X3)
22	62	M	I	47, XY, del(5)(q12q13), del(6)(q23), del(11)(q23) [2]/ 47, XY, del(6)(q23), +11, del(11)(q22), +12, −14 [3]/ 46 - 47, XY, del(6)(q23), del(7)(q32) [2], +11 [5], del(11q) [5], +12 [3], −14 [3], del(16)(q22) [2], +21 [3] [cp11]	nuc ish(D11Z1X2, *ATM*X1), (D6Z1X2, *MYB*X1)
23	45	M	0	46, XY [16]	Negative
24	52	F	III	46, XX, del(15)(q22.3q24) [14]/46, XX [3]	Negative
25	72	M	II	47, XY, +12 [16]/47, XY, +1, +12[cp2]/46, XY [1]	nuc ish(CEP12X3)
26	45	F	II	46, XY, t(14;18)(q32;q21) [4]/46, XY	Negative
27	64	M	IV	46, XY, del(6)(q21q23) [5]/46, XY, del(6q) [4], t(?;7)(?;p22) [4], t(?;12)(?;p13) [2] [cp6]/46, XY [6]	nuc ish(D6Z1X2, *MYB*X1)
28	61	F	IV	46, XX, del(13)(q12q14) [16]	nuc ish(D13S319X1, 13q34X2)
29	54	F	III	46, XX, t(?;14)(?;q32) [10]/46, XX [10]	Negative
30	55	M	II	46, XY, del(14)(q11.2q24) [4]/46, XY [6]	Negative
31	71	M	III	45, XY, −13 [3]/45 - 46, XY, del(1)(q11) [2], del(4)(p15.2) [2], t(?;10)(?;q24) [2], −13 [5], +19 [2][cp8]/46, XY [9]	nuc ish(D13S319X1, 13q34X1)

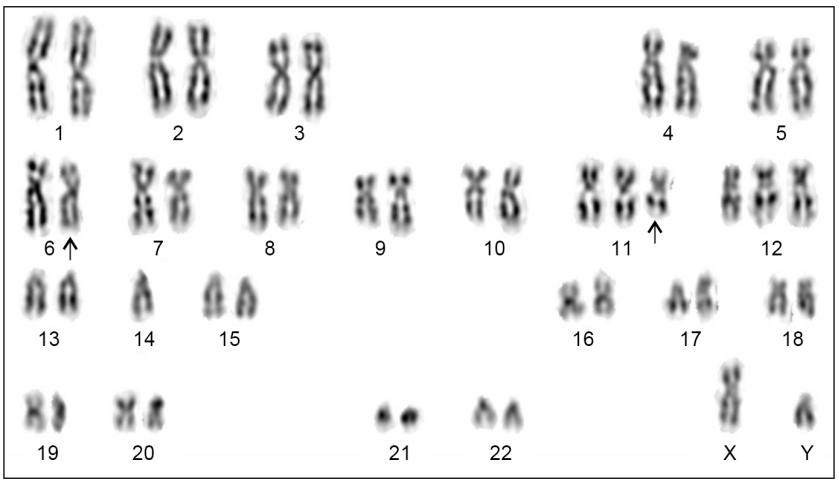

Figure 5. Conventional karyotype of a case with del(6q23) and del(11q22) by FISH shows complex karyotype with del(6)(q23), +11, del(11q)(q22), +12, −14.

In a cohort of 31 cases, 7 cases (24%) had complex karyotype. All cases with complex karyotype had targeted FISH aberrations like del(13q), +12, del(11q), del(17p), del(6q) and also additionally displayed numerical as well as structural abnormalities like deletions, and unbalanced translocations (**Table 5**, **Figure 5**). A significant association was observed between complex karyotype and coexistence of ≥2 FISH markers ($p = 0.009$) and del(11q22) &/or del(17p) ($p = 0.03$). A significant association was found between non-complex karyotype and cases with negative FISH markers ($p = 0.01$) (**Table 6**).

4. Discussion

The incidence of targeted FISH abnormalities was 71% in our series of 671 patients of B-CLL and that was consistent with the range (60% - 80%) of reported studies [10]-[12] [17] [22] [30] [31]. As summarized in **Table 1**,

Table 6. Correlation of FISH markers with complex and non-complex karyotype (n = 31).

FISH Genetic Groups	Complex Karyotype (%)		Non-Complex Karyotype (%)		P value
	Positive	Negative	Positive	Negative	
del(13q)/-13	0	13(37)	4(100)	22(63)	0.281
Trisomy 12	1(13)	12(39)	7(88)	19(62)	0.229
del(11q22)	1(33)	12(33)	2(67)	24(67)	1.0
del(17p13)/-17	2(100)	11(30)	0	26(70)	0.105
del(11q)&/or Abn(17p)	5(71)	8(25)	2(29)	24(75)	0.03
del(6q23)	0	13(34)	1(100)	25(66)	1.0
≥2 aberrations	4(100)	9(26)	0	26(74)	0.009
Negative	0	13(45)	10(100)	16(55)	0.01

del(13q)/-13(43%) was most common, followed by trisomy 12(19%), del(17p)(15%), del(11q)(13%) and del(6q)(8%) [10]-[12] [21] [22] [31]. Although reported incidence of del(17p) is 4% - 15%, the frequency in our series of del(17p) was towards higher side 15% than del(11q22)(13%). The correlation of Rai staging with 17p deletion showed no significant association of del(17p) with stage of the disease in B-CLL which may indicate that del(17p) may not necessarily be associated with disease stage, rather it occurs frequently in progressive disease [11] [12] [18] [21] [32]. In the present study, the identification of progressive vs non-progressive disease and treatment response with cytogenetic findings was not possible due to unavailability of clinical follow up.

A biallelic 13q deletion with incidence of 9% - 10% has been observed in other studies. We did not find correlation between mono/biallelic 13q deletion with age and stage of disease. The clinical impact of an biallelic 13q deletion is controversial [11] [33] [34].

The predominant clone of abn(13q) in cases with coexistence of ≥2 abnormalities is consistent with reported studies, which confirmed that abn(13q) is a most common genetic event [10] [12] [17] [21] [31]. The frequencies of all FISH markers as a sole aberration and in combination with other recurrent markers revealed that del(11q22) and del(17p) occur frequently in combination with other recurrent FISH marker/s rather than sole aberration. We found correlation of Abn(13q) with lower age and lower stage of disease. There was no association of high risk markers del(11q) and del(17p) with either variable age or stage of the disease, whereas group of coexistence of ≥2 abnormalities was associated with Rai stage III, IV. Study by Quijano *et al.* [11] found higher proliferative index in B-CLL patients with co-existence of del(13q) & del(17p). The review of literature emphasized that del(17p), a high risk progressive group commonly seen in association with high risk factors like unmutated *IgVH*, CD38 positivity rather than stage of disease [10] [11] [13] [14] [18] [22] [32] [35].

The frequency of *IgH* translocation in our study was 7%, is consistent with reported studies 4% - 9% [9] [27]-[29]. Overall, 62% of *IgH* translocation +ve cases belonged to atypical CLL in which CD38 was present in 38% of cases. Translocation (14;18) and t(14;19) were most predominant (64%). The review of literature has also shown involvement of *BCL2* and *BCL3* as commonly affected partner genes [25] [27]-[29]. The association of *IgH* translocation with trisomy 12 followed by del(13q) as additional aberrations and the clone size of aberrant nuclei with *IgH* translocation, +12, del(13q) revealed that *IgH* translocation may be a primary event . Previously reported studies on *IgH* translocation in B-CLL have shown an association of *IgH* translocation with unmutated *IgVH*, ZAP70 expression, CD38 positivity, atypical nuclear morphology, atypical immunophenotype [7] [27]-[29].

Conventional karyotyping with stimulated cultures with CPG Oligonucleotide with IL-2 as well as TPA found to be very efficient strategy as compared to FISH, enabled to detect targeted FISH aberrations as well as non-targeted comprehensive chromosomal aberrations in 97% of cases. Overall, 71% of cases had heterogeneous chromosomal abnormalities with affected loci other than FISH targeted loci which were present as either sole or as additional abnormalities along with FISH markers. We could efficiently identify large 13q deletion, del(13)(q12q14) & del(13)(q14q22) in two cases, where FISH failed to detect these non-targeted large deletions. Large deletions covering 13q12q14/q14q22 which fall in class II deletion cover RB1 and many additional genes,

which may drive disease progression probably through involvement of novel genes pathways [36] [37]. The complex karyotype frequency of 24% falls in the range of 15% - 25% in the studies reported earlier [7]-[9] [14] [25] [26]. The numerical abnormalities and structural abnormalities affecting various chromosomal loci in complex and non-complex karyotypes were heterogeneous. Some of these abnormalities like +6, +11, −18, +19, del(1)(q11-q25), del(4p15), del(7)(q22-23), del(13)(q14q22), del(18p11), t(10q24), t(11q21-q23), t(12p13), t(14q32), t(14p11) although not recurrent but have been described previously in B-CLL [7]-[9] [23] [25]. A large scale karyotyping data is required to check the recurrence of the abnormal chromosomal loci in our study.

The complex karyotype displayed FISH targeted recurrent aberrations like del(13q14), +12, del(17p13), del(11q22), del(6q22) as well as non-targeted numerical and structural abnormalities, which were also detected in non-complex abnormal karyotypes. The significant correlation of complex karyotype with del(11q22) and/or del(17p) and also with FISH subgroup, coexistence of ≥2 abnormalities supports and reflects the fact that B-CLL with complex genome may be a consequence of progressive genomic instability with poor prognosis. This is supported by recent findings that showed complex karyotype with association of poor prognostic markers like del(17p), unmutated *IgVH*, decreased event free survival, chemo refractory to standard Fludarabine and also targeted Ibrutinib-based regimens [9] [32] [35] [38]. Recently, Thompson *et al.* [32] reported that complex karyotype is an independent powerful predictor of outcome of targeted Ibrutinib-based regimen as well as stronger predictor of biological behavior than del(17p).

Recently, Sanger sequencing and next generation sequencing have identified new genomic abnormalities such as *NOTCH1*, *SF*3*B*1, and *MYD*88 & *BIRC*3 mutations along with *TP*53 deletion/point mutation. *NOTCH*1 mutation is associated with higher risk of Richter Syndrome transformation. *SF*3*B*1 mutation is associated with chemo resistance to alkylating/Fludarabine therapy. *BIRC*3 mutation is associated with chemorefractory, relapsed disease and found to be very high risk, independent prognostic marker [15] [16] [18] [19] [30] [39] [40]. These mutation markers along with integrated cytogenetic findings will contribute in better refinement of prognostic groups in B-CLL.

5. Conclusion

In conclusion, our study shows that interphase FISH with integration of conventional karyotyping is powerful strategy, able to identify not only recurrent targeted abnormalities but also genomic complexity with clinical significance that helped identification of additional prognostic subclasses. The point mutation markers along with integrated cytogenetic findings will contribute in better refinement of prognostic groups and help design risk-adapted treatment strategies in B-CLL.

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