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Importance of Cytogenetic and Molecular Cytogenetic Markers Related to prognosis in Chronic Lymphocytic Leukemia

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ABSTRACT: Characteristic chromosome abnormalities in patients with chronic lymphocytic leukemia (CLL) have been shown to provide important prognostic information. Karyotyping and Fluorescence *in situ* hybridization (FISH) currently used in clinical diagnostics of CLL are targeted tests aimed at specific Cytogenetic marker. The aim of this study was to understand the role of the Cytogenetics and molecular Cytogenetic markers in Chronic Lymphocytic Leukemia through Cytogenetics techniques, i.e. Conventional Cytogenetics and FISH in a clinical diagnostic setting. Karyotyping and FISH were performed in all 15 CLL patients and compared with international prognostic scoring criteria. Here, we conclude that the clinical significance of cytogenetic examination in CLL is apparent. That most of patients with CLL show clonal Chromosomal aberrations (CAs) has become clearer with the ongoing approach of more sensitive detection methods. In CLL, there is a positive relationship between the presence of CAs and progressive disease, showing a prognostic role for cytogenetic investigations. The prognostic relevance of these Cytogenetics alterations requires further evaluation in prospective of targeted therapy.

Key words: chronic lymphocytic leukemia, Cytogenetic markers, Fluorescence *in situ* hybridization (FISH), Karyotyping, International Prognosis Scoring System (IPPS), Clonal chromosomal aberrations (CAs)

1. INTRODUCTION

Chronic lymphocytic leukemia (CLL) is a heterogeneous disease, running an indolent course in some patients and a clinically aggressive course in others. Chronic lymphocytic leukemia (CLL), the most common leukemia diagnosed in adults from Western countries, is characterized by a monoclonal population of mature activated B lymphocytes that usually express CD5+ and CD23+.Many host- and tumor-related features with prognostic and/or predictive value have been identified over the years, assisting in the stratification of patients into subgroups with distinct clinical course and response to treatment B-cell. CLL exhibits a highly heterogeneous clinical course, with overall survival rates varying from several months to decades. Most patients diagnosed with CLL can survive for many years, but in a subset of patients the course progresses more rapidly and is fatal despite aggressive treatment. Conventional cytogenetic analyses result in the identification of genetic abnormalities in a relatively low percentage of patients, due to the low in vitro proliferative potential of CLL cells. Comprehensive prognostic indexes including clinical and biological parameters were published .Prognostic/predictive factors include advanced stage, positivity for CD38, ZAP70 and CD49d, the unmutated configuration of the variable region of the immunoglobulin heavy chain (IGHV) gene and specific cytogenetic lesions revealed by fluorescent in situ hybridization (FISH). Recent studies also demonstrated the independent negative prognostic impact of

mutations of several genes including TP53, NOTCH1 and SF3B1.The most common molecular-cytogenetic techniques currently used to detect these abnormalities in CLL are fluorescence in situ hybridization (FISH) IGHV, karyotype aberrations and multiplex ligation dependent probe amplification (MLPA) .We therefore investigated whether an extended genetic characterization, including karyotype analysis and FISH analysis, could predict outcome in high-risk CLL patients.

Pathophysiology:

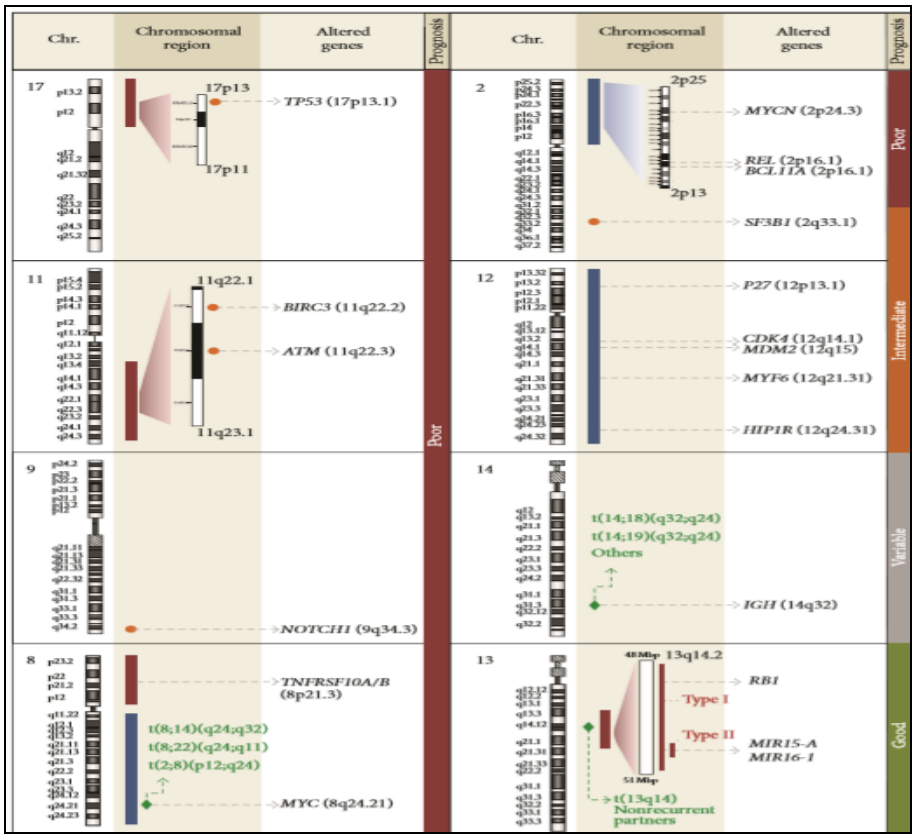
It is characterized by proliferation of small, abnormal, mature B lymphocytes, often leading to decreased synthesis of immunoglobulin and depressed antibody response. The number of mature lymphocytes in peripheral blood smear and bone marrow are greatly increased.

Incidence:

Adults > 50yrs, 5% cases: 40-50 yrs,

10% cases: 10-30 yrs, Peak incidence: 60-80yrs & Male: Female ratio = 2:1

1.1 Cytogenetic aberrations with known Prognostic value of CLL



1.2 Risk & Molecular significance of Prognostic markers

Prognostic Marker	Low Risk	High Risk	Molecular Significane
CD38	Negative	Positive	Signals cell Activation
ZAP-70	Negative	Positive	TK involved with cell activation
IgVH mutation	Mutated	Unmutated	Somatic hypermutation

1.3 International prognosis scoring system (IPSS):

It is used to help assess the severity of a patient, which is based on the IPSS score, the patient's history, his/her personal observations, the physician will design a treatment plan to address the CLL. It looks at factors of blast cells in bone marrow, Cytogenetics (Chromosomal changes), Cytopenias (Level of haemoglobin, absolute neutrophil count, platelet count). In the original CLL-IPI scoring system, patients are segregated into four risk categories: 1) Low (Score= 0-1, Chances of survival for 5 years= 95%).

2) Intermediate (Score= 2-3, Chances of survival for 5 years= 82%)

3) High (Score= 4-6, Chances of survival for 5 years= 68%)

4) Very high (Score= 7-10, Chances of survival for 5 years= 19%)

Factors	Abnormalities	Values	IPSS
Blast cells in bone marrow (Percent)		≤2	0
		>2 - <5	1
		5 - 10	2
		>10	3
Cytogenetics	• del(13q)	Good	1
	• 14q- t(14:18) t(14:19)	Inter-mediate	3
	• Trisomy-12 • del(17p)	Poor	4
	• del(11q) • del(6q) • del(2p) • del(8p)	Very poor	7
Cytopenias	Haemoglobin (g/dL)	≥10	0
		8 - <10	1
		<8	1.5
	Absolute neutrophil count(ANC)	≥0.8	0
		<0.8	0.5

	Platelet count(µL)	≥100	0
		50-<100	0.5
		<50	1

(International Prognosis Scoring table)

2. MATERIALS & METHODS

2.1 Ethical Clearance

The study was approved by the Institutional Ethics committee of inDNA Life Sciences PVT. LTD.

2.2 Sample Collection

Sample was collected from bone marrow in a Sodium Heparin vial (Green) from 15 patients (age 14-78years) by an authorized clinician diagnosed with CLL after obtaining prior informed consent.

2.3 Karyotyping

Required reagents for 24,48,72hr BM culture:

1) Bone marrow sample

2) Complete media (RPMI1640): RPMI1640 (Plain media) + 8-10% of Foetal Bovine Serum (FBS) + 1% of antibiotics & L-glutamine

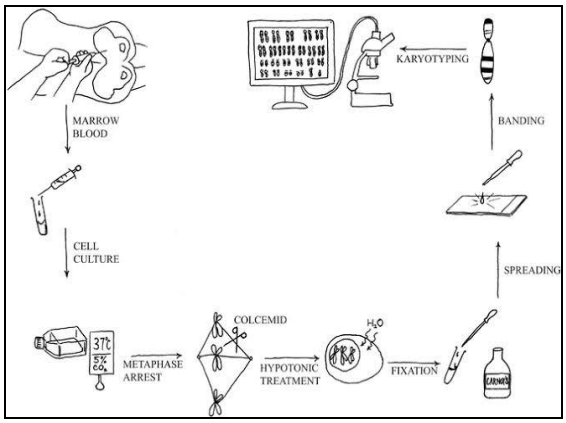
3) Colcemid

4) Hypotonic solution (KCL) (0.07M): 25ml distilled H_2O + 0.14gm KCL

5) Carnoy's fixative: Addition of Methanol & Acetic acid in 3:1 respectively.

But in 0hr. culture complete RPMI1640 is not required. Involved steps are culture initiation by taking cultured BM, culture harvesting, slide casting, staining & slide observation.

It has been reported to range from 14% to 40% for +12q as isolated abnormality, 10% to 32% for 11q-, 11% to 18% for 13q, 3% to 27% for 17p-, and 2% to 9% for 6q-, depending on the stage of the disease.



(Fig-1: Process of Karyotyping)

2.4 Fluorescence *in Situ* Hybridization (FISH)

Required reagents for Metaphase& Interphase:

1) Saline Sodium Citrate buffer (0.4X): 49ml distilled H_2O + 1ml of 20X SSC buffer (Pre prepared stock solution) to make 50ml solution

2) Saline Sodium Citrate buffer (2X): 45ml distilled H_2O + 5ml of 20X SSC buffer (Pre prepared stock solution) to make 50ml solution

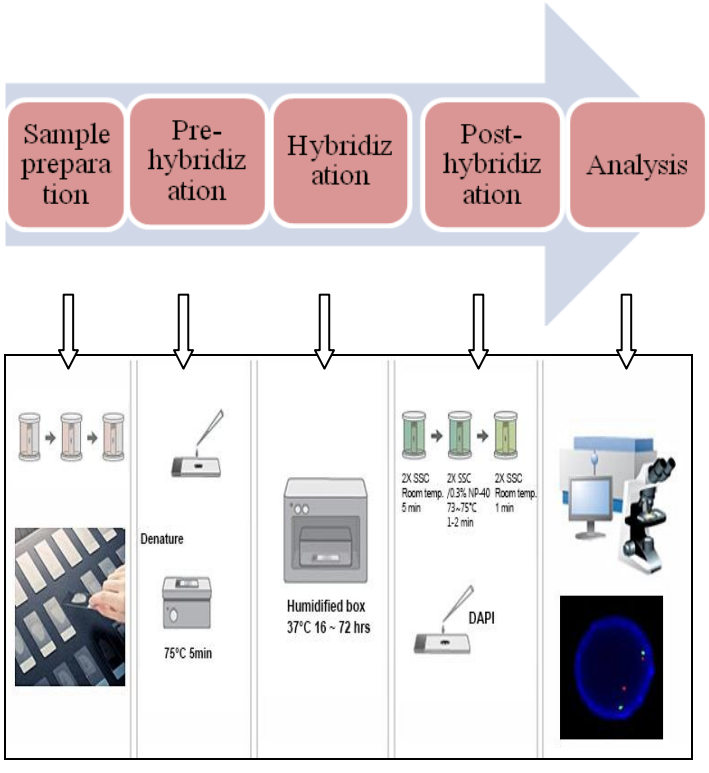
3) Distilled water

4) Tween 20

5) Fluorescent labelled probes

6) DAPI counter stain

Involved steps are sample preparation, pre-hybridization, hybridization, post- hybridization & microscopic analysis. The following commercially available probes were used for FISH: ATM (11q22), centromere 12, D13S319 (13q14) and TP53 (17p13). FISH was performed according to the manufacturer's specifications. At least 100 interphase nuclei were scored by two independent investigators. The cut-off values for both gains and losses were determined by statistical evaluation of FISH results from control tissues: for each probe the mean + 3 standard deviations of false positive nuclei was taken as the cut-off level. Cytogenetic abnormalities can be detected in up to 90% of the patients by interphase FISH.

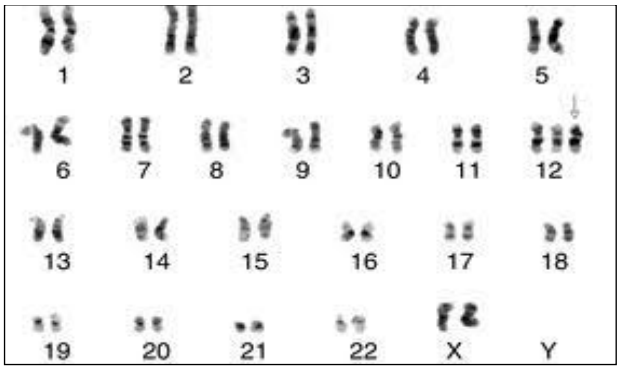


(Fig-2: Steps of FISH)

3. RESULT

3.1 Karyotype Analysis

The collected bone marrow specimens were processed for 0hr, 24hr, 48hr and 72hr unstimulated chromosome cultures. The analysed karyotypes appeared numerically and structurally normal for 13 cases, trisomy of chromosome-12 in two cases were found.

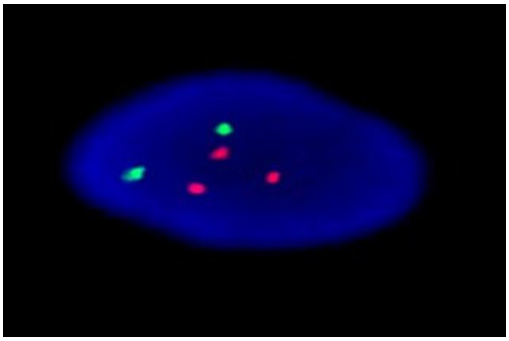


(Fig-3: Trisomy of chromosome no-12)

3.2 FISH Analysis

The bone marrow specimen was processed for interphase FISH for CLL FISH probe panel. Upon interphase FISH analysis of the uncultured marrow cells. The provided bone marrow specimen was appeared normal for 8 cases, trisomies of chromosome-12 in 4cases were found.

A. 02 copies of 5 chromosome 12 (red)



(Fig-4: Representative interphase FISH image)

4. DISCUSSION

The median overall survival for CLL patients is 9 years. The presence of clonal chromosomal abnormalities predicts for a shorter overall survival time. Specifically, complex abnormalities, increased percentages of abnormal metaphases, abnormalities of the long arm of chromosome 14, and trisomy 12 have been associated with shortened survival. Although cytogenetic abnormalities are prognostically important in CLL, approximately 50% to 70% of patients have either a diploid karyotype or insufficient metaphases for analysis. In this study of CLL, FISH detected a higher incidence of trisomy 12 than conventional cytogenetic. A decreased overall survival was suggested in the study of Anastasia, but was not statistically significant. With the use of FISH, we were able to detect 2.6 times as many cases of trisomy 12 were detected with conventional cytogenetic analysis in the samples studied with both techniques. This was primarily due to the detection of trisomy 12 in samples that were classified as diploid or had insufficient metaphases for conventional analysis. In cases of trisomy 12 identified with FISH that were originally classified as cytogenetically diploid or as having insufficient metaphases, dividing normal T cells may have led to misleading result. About one-fourth of patients with trisomy 12 had a low percentage of trisomic cells, suggesting clonal evolution. This finding may also suggest that trisomy 12 is a secondary event in at least a proportion of the patients. If trisomy 12 was present in more than 50% of cells by FISH, it was more likely to also be detected with conventional Cytogenetics, but in none of the samples with less than 50% trisomic cells was cytogenetic analysis successful. So, Genomic variations in CLL are important independent predictors of disease progression and. These discoveries may help further characterize CLL as per their prognosis.

5. CONCLUSION

The clinical significance of cytogenetic examination in CLL is apparent. That most of patients with CLL show clonal Chromosomal aberrations (CAs) has become clearer with the ongoing approach of more sensitive detection methods. **A low mitotic index has generally been a restricting factor for conventional cytogenetic examination in CLL. In CLL, there is a positive relationship between the presence of CAs and progressive disease, showing a prognostic role for cytogenetic investigations**. Genomic variations in CLL are important independent predictors of disease progression and. These studies may help further characterize CLL in to subgroups as per their prognosis, especially early in the course of sickness, that possible will have suggestions for the plan of future risk adapted treatment procedures.

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