

# CYTOGENETICS

## **Comparative Genomic Hybridization study of myelodysplastic syndrome**

***Year of commencement*** : ***2004***

***Year of completion*** : ***Ongoing***

### **Background :**

Advancement in cytogenetics technology using fluorescence in situ hybridization (FISH), provided to detect aneuploidy and micro chromosomal rearrangements in interphase and metaphases. However, many cases of AML and MDS have structurally altered chromosomes as a part of a complex karyotype, which cannot be fully identified by cytogenetics analysis and FISH. Identification of chromosomal regions involved in rearrangements and net gains and losses of chromosomal copy numbers could be of considerable clinical importance. We had carried out a study (1999-2004) in MDS patients using conventional cytogenetics revealed only 40% of chromosomal abnormalities in MDS patients. The present study designed to apply comparative genomic hybridization (CGH) to MDS patients submitted to routine cytogenetics analysis to investigate whether this approach would identify any new chromosomal regions in the development of MDS.

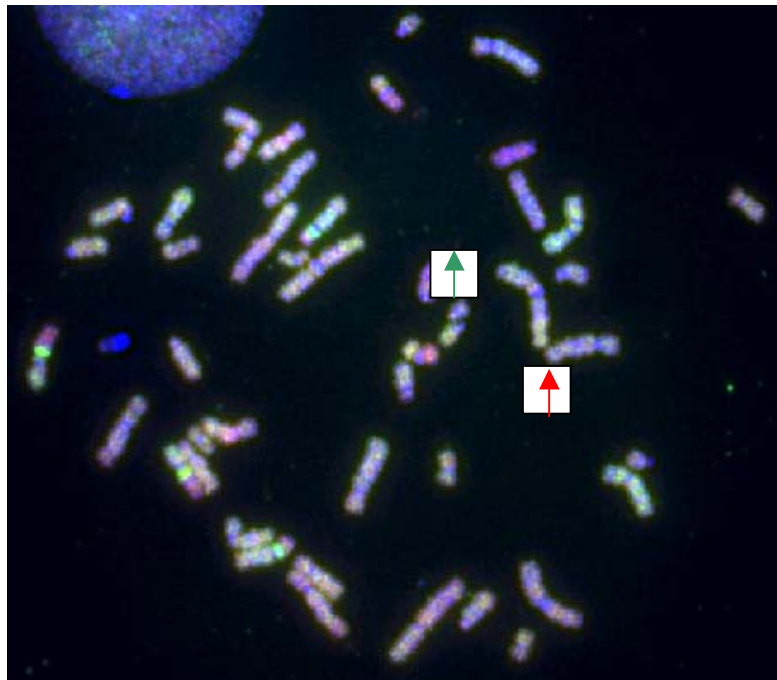
During one year period forty two suspected MDS patients were studied. The patients details regarding age, sex, occupation, living environment etc., were recorded in the proforma. Chromosomal preparations obtained from Bone marrow samples using standard procedure were subjected to GTG – banding. At least 20 metaphases were analysed in each case and karyotyped according to ISCN (2000). FISH analysis was carried out using chromosome 5 and 7 centromeric and locus specific probes.

CGH experiment : Metaphase spreads were prepared according to standard protocols from PHA – stimulated peripheral blood lymphocytes from a karyotypically normal male and females. The peripheral blood collected from MDS and controls in EDTA used for DNA extraction. The test – DNA was labeled with fluorescence – 12 dUTP and control DNA was labeled with Cy3 (red) according to the standard nick-translation method. The probe size was within 500 bp by adjusting DNA's concentration. A high concentration of (40mg) cot-1 DNA was used to suppress the repetitive sequences. The chromosomal DNA and test and control DNA was denatured at 75C in 70% formamide for 5 minutes, then applied to denatured target metaphases on dry slides were stained with DAPI. The analysis was done using fluorescence microscopy and IMSTAR image system. The experiment was standardized with different DNA concentrations and test DNA with known chromosomal imbalances.

### **Results :**

The MDS patients were sub grouped according to FAB classification and majority (50%) of patients were RA subgroup. The cytogenetics analysis using GTG-banding and FISH revealed 57.14% chromosomal abnormality and high frequency of chromosomal aberrations were detected in RA subgroup (Table 1). The CGH experiment was standardized (Fig 1) using different steps and was carried out in 10 MDS patients. Six of the 10 MDS cases (60%) showed copy number changes, whereas the remaining 4 (40%) were normal. DNA copy gains were more frequent than losses. The gains on chromosomes were 1p, 1q, 5p, 18q, 21q and losses were on 2q, 5q, 7q and 17q. However these regions should be mapped using locus specific probes.

**Fig.1 CGH showing DNA copy number variation as gains (green) and losses (red) on metaphase.**



**Table : Frequency of chromosomal abnormalities in MDS patients**

<b>Sr. No.</b>	<b>Subgroups</b>	<b>No.</b>	<b>%</b>	<b>Chromosomal abnormality No.</b>	<b>%</b>
1.	RA	21	50.00	13	54.17
2.	RAEB	8	19.05	5	20.83
3.	RAAB – t	7	16.67	4	16.67
4.	CMMML	4	9.52	2	8.33
5.	RARAS	2	4.76	-	-
	Total	42		24	57.14%

## **Cytogenetic study of Acute lymphoblastic leukemia in young**

***Year of commencement: 2004***

***Year of Completion : Ongoing***

### **Objectives:**

1. To detect the chromosome abnormalities by conventional cytogenetic method (GTG- banding).
2. Molecular cytogenetic evaluation of cryptic and complex translocations by FISH and CGH study in all the patients.
3. Correlation of cytogenetic abnormalities with immunophenotyping and other hematological parameters.

Cytogenetic study was carried out in 30 de novo ALL patients who were below 15 years of age. All clinical details were recorded in our proforma. Chromosome preparation from bone marrow was done by direct method. The cultures were set up with B and T cell specific stimulants on blood and bone marrow samples whenever it was required. The EDTA samples of the patients were also collected for the CGH analysis. Chromosome analysis was done on GTG banded metaphases and FISH analysis of 12 B-ALL patients were done by using TEL/AML probe. Among these 30 patients 19 (63.3%) were found to be cytogenetically abnormal including novel chromosome aberrations t(12;20), t(2;19) and the type of chromosomal abnormalities are shown in table.

**Table: Chromosomal abnormality in young ALL patients**

<b>Diagnosis</b>	<b>No. of Patients</b>	<b>Type of Chromosome abnormalities</b>	<b>No. %</b>
B-ALL	18 (60%)	Hyperdiploidy > 50 Chromosome	1
		Hyperdiploidy with TEL/AML1	2
		+ve	1
		Hyperdiploidy with t(12;20) (q26;	1

		p11) Tetraploidy Hypodiploidy <45 chromosome Extra marker chromosome TEL/AML1 positive	61.11% 2 2 2
T ALL	– 11 (36.6%)	Hyperdiploidy > 50 chromosome Hypodiploidy < 45 chromosome Near haploidy del (9)(p22–ter) inv (9)(p23 q21) t (10;14) (q24; q11) del(7)(p12→ ter), + mar	1 1 1 1 63.63% 1 1 1
NK Cell	1 (3.3%)	47,XYX/ (2;19)(p13;p13),add(9)(p24)	t 1
<b>Total</b>	<b>30</b>		<b>19</b> <b>(63.33%)</b>