RESEARCH COMMUNICATION

Chromosomal Breakage in Myelodysplatic Syndrome

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Abstract

The myelodysplastic syndrome (MDS) represents a group of clonal hematological disorders characterized by progressive cytopenia reflecting defects in erythroid, myeloid and mega karyocytic maturation. The incidence of MDS is greter in older age groups. Detailed studies on MDS from India are not available. Cytogenetic study using GTG-banding and FISH revealed 54.5% clonal chromosomal abnormalities. We have carried out chromosomal breakage study from peripheral blood cultures induced with mitomycin C, in karyotypically normal MDS (49) and 15 (30.6%) showed significant ($p < 0.001$) increase in chromosome damage compared to controls. Among 22 occupationally exposed MDS, 6 (27.3%) showed a high frequency of chromosome breakage while in the non-exposure (n=27) group, high chromosome breakage was noted in 9 (33.3%) MDS patients. Our results suggest that the high chromosome damage may be due to acquired Fanconi anemia which leads to multiple defects in chromosomes and clonal chromosome anomalies.

Key Words: Myelodysplastic syndrome - chromosome aberrations - breakage - Fanconi anemia - occupational exposure

Asian Pacific J Cancer Prev, **8**, 151-154

Introduction

Myelodysplastic syndrome (MDS) represents a group of clonal haematological disorders characterized by progressive cytopenia reflecting defects in erythroid, myeloid and megakaryocytic maturation (Bennetttt et al. 1983). Though MDS in the western countries are mainly found in the elderly population and rarely in the pediatric age group; this disease is being increasingly seen in young adults in India. Cytogenetic study plays a important role in the diagnosis of primary and secondary MDS . Clonal nonrandom chromosomal changes ranging between 23% and 78% have been reported in MDS patients from the United States, Japan and Europe (Ayraud et al 1983;Gold et al 1983; Knapp et al. 1985; Jacob et al. 1986; Larripa et al. 1987; Gyger et al. 1988; Horke et al. 1988; Muslilova et al. 1988; Yunis et al. 1988; Pierre et al. 1989). However only a couple of studies are reported from India (Ahmed et al 1993; Kadam P et al. 1995). Yunis et al. (1988) have reported a much higher frequency (79%) of clonal abnormalities in consecutively investigated MDS patients using high – resolution G-banding methods. The most commonly involved chromosomal changes in primary MDS have been monosomies 5,7, trisomy 8, deleltions 5q, 7q, 9q and 20q (TMIC 1987; Le Beau and Onley 2001). Although a large number of reports on the risk factors of leukemia have been published , little is known about the risk factors of the MDS (Levine and Bloomfield 1992 Vogel and Fisher 1993). The occupational exposure to chemicals, radiations and habits such as smoking, alcohol consumption are reported to be associated with development of MDS (Nisse et al. 2001). The present study elaborates the range of cytogenetic anomalies found in Indian patients and occupational data on these patients is included. An increased sensitivity to chromosome breakage of peripheral blood lymphocytes of these patients when exposed to mitomycin C is also presented.

Materials and Methods

One hundred and forty five (145) patients attending Haematology clinic, KEM Hospital, Mumbai, India diagnosed according to WHO criteria and was the material for the study. K.E.M hospital is one of the biggest tertiary care center in Mumbai, India. Almost 0.5 million patient attends the hospital inpatient and outpatient facility every year. Clinical, hematological and environmental and occupational exposure data were obtained from these patients using a questionnaire prepared for this purpose. Bone marrow (BM) and peripheral blood (PB) was obtained from all the patients on admission with prior consent. The study was conducted for a period of six years (2001-2006).

Cytogenetic study

The BM samples collected in F-10 nutrient media with 20% fetal bovine serum (FBS) were arrested with colcemid using direct and 24 hours culture methods. The cultures treated with 0.075 M hypotonic solution (KCl) were fixed with 3: 1 methanol and acetic acid. The chromosomal preparations obtained by droping on pre chilled slides were subjected to GTG banding.The chromosomal analysis was done from at least 20 metaphases from an individual and karyotyping was done

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Table 1. Chromosome Aberration Frequencies in MDS Subtypes

S.no	Subtype	No.	Chromosome aberration	
			No.	%
1	RA	55	26	47.3
2	RAEB	41	24	58.5
3	RAEB-t	30	24	80.0
4	CMMOL	10	3	30.0
5	RARS	9	2	22.2
	Total	145	79	54.5

according to International system for chromosome nomenclature (ISCN) 1995 . Fluorescence in situ hybridization (FISH) analysis was carried out using centromeric probes (5,7,and 8) and locus specific probes (5q33, 7q31-33) according to manufactures instructions (Vysis,Abbott,Germany).

Chromosome breakage study

Peripheral blood lymphocyte cultures stimulated with phytohaemagglutinin (PHA), were treated with 40 ng/ml mytomycin C (MMC) for 72 hrs in the dark at 37C (degree) and cultures were then harvested after a 30 minutes exposure to colcemid . After a 30 minutes treatment with 0.075 M KCl, the cells were fixed with 3:1 mixture of methanol and acetic acid, respectively. Wet mount slides were prepared, dried on hot plate and stained with Giemsa stain. At least 30 metaphase cells from each culture were scored for chromosome breaks and radial forms. Gaps, breaks and other rearrangements were recorded on a chart. Breaks were considered to be equal to or greater than a chromatid width apart and gaps were less than a chromatid width apart. Each chromatid or chromosome break was scored as one break. In case of triradial, tetraradials forms of breakage considered as 2 and 3 breakages respectively. Chromosome breakage score was expressed as breakage per metaphase and breakage per aberrant metaphase. During this period 25 healthy adult peripheral blood lymphocytes were similarly cultured with MMC and the chromosome breakage was compared with the patients using Student's *t*- test.

Results

The chromosomal analysis from BM samples using GTG-banding and FISH revealed 54.5% chromosomal abnormality out of 145 patients studied. The frequency

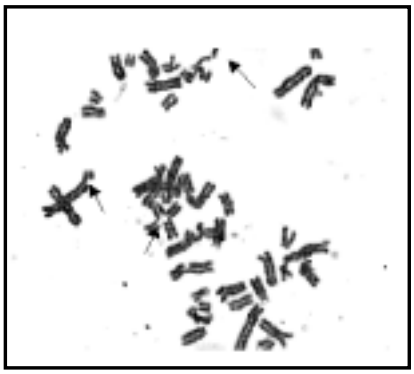

Figure 1. Metaphase Showing Chromosome Breakage in an MMC Induced Peripheral Blood Culture

of chromosomal abnormality in MDS subgroup is presented in Table 1. The majority of patients were in RA (37.93%), RAEB (28.27%) and RAEB-t (20.69%). Out of 66 karyotypically normal MDS, the chromosome breakage study using MMC induction in cultures was carried out from 49 MDS patients. 15 (30.61%) MDS showed significantly ($p<0.001$) high frequency of chromosome breakage compared to controls (table. 2) (Figure). The data on occupational and living environment of 49 MDS patients is presented in table 3. Out of 49 karyotypically normal MDS, 22 (44.89%) had history of occupational exposure and high frequency of chromosomal breakage was detected in 6(27.27%) MDS Patients. The chromosomal breakage also detected in 9(33.33%) non-exposure MDS out of 27 patients tested.

Discussion

The cytogenetic changes frequently reported in MDS and common chromosome aberrations reported to be monosomies 5,7, and trisomy 8. In our study with combination of GTG-banding and FISH , the chromosomal aberrations detected in 79 (54.48%) MDS patients out of 145 studied. During our study spontaneous chromosomal damage was noticed in some group of patients, who were karyotypically normal. We have designed a study to estimate chromosomal breakage frequency in karyotypically normal MDS. Among 66 karyotypically normal patients, chromosomal breakage study was carried out in 49 patients and none of these patients hold any physical, cytomorphological and family history of Fanconi anemia (FA). Significant ($p<0.001$) increase in chromosome breakage (up to 5 times more than normal

Table 2. Chromosome Breakage Frequency in Karyotypically Normal MDS Patients

S.No.	Subgroup	Patients Chromosome breakage		Total metaphases scored	Metaphases with breaks		Total chromosome breakage	Chromosome break/ metaphase	Chromosome break/aberrant metaphase
		No.	%		No.	%			
1	RA N=26	9	34.6	290	215	74.1	1121	3.87	5.21
2	RAEB N=17	5	29.4	175	128	73.1	773	4.42	6.04
3	RAEB-t	1	16.7	35	23	65.7	124	3.54	5.39
Total	49	15	30.6	500	366	73.2	2018	4.03*	5.51*
Controls	25	-	-	750	52	6.9	68	0.09	1.31

*$p<0.001$

Table 3. Occupational Exposure and Chromosomal Instability

S. No	Exposure	MDS		Chromosome Breakage	
		No.	%	No.	%
1	Agriculture (pesticides etc.)	8	16.32	3	6.12
2	Industrial (steel,benzene, paints, polyethelene)	5	10.21	2	4.08
3	Automobiles, Petroleum, etc.	6	12.24	1	2.04
4	Living Environment (Industrial)	3	6.12		
5	Non-Exposures	27	55.11	9	18.36
	Total	49	100.0	15	30.61

control) were found in 15 (30.61%) cytogenetically normal MDS patients , compared to that of healthy controls (table 2). Etiological factors of the MDS are largely unknown, with the exception of alkalyting agents, ionizing radiation and benzene (Farrow et al., 1989; Goldberg et al., 1990; Ido et al., 1996). Some other risk factors have been suggested by the few epidemiological studies reported (solvents, ammonia, exhause gases, metals, pesticides, alcohol) (West et al., 1995). In our study 22 (44.9%) MDS had a history of occupatioanal exposure (table.3). A high frequency (50%) of MDS occupation was industrial and these were exposed to benzene, steel, polyethylene, petroleum etc. However, only 3(27%) MDS shown chromosomal breakage.

In case of MDS exposed to pesticides (36%)(occupation was agriculture), 37% found to had chromosomal breakage, which suggests that the occupational exposure (pesticides or industrial chemicals) may have role in chromosomal damage and developing the disease. Indian agriculture at present is heavily dependent on chemical fertilizers and pesticides. Most of the chemical fertilizers like ammonium phosphate, potassium nitrate, calcium phosphate and urea are not known mutagens or carcinogens. However, pesticides used in India are organophosphorous, carbonate and organo-chlorine compounds. Occassional mercurial anti fungal agents are also used. Indian farmers often do not take enough precaution to protect themselves from exposure of these pestcides and acute poisoning leading to death is also not uncommon. Often these patients kept pestcides within exposure to other members of the household. However detailed study in large group of patients is essential to correlate the occupational exposure to the disease.

Interestingly in our study, 33% MDS patients without any occupational exposure history exhibited a high frequency of chromosomal breakage. This tempted us to speculate that in some MDS patients an acquired Fanconi like lesion may be the initial genetic abnormality. Subsequently manifest cytogenetic abnormality in MDS patients may stem from the defective repair capability due to the initial lesion and subsequent clonal evolution. This speculation is strengthened by the fact that deletion of Fanconi syndrome associated gene has been demonstrated as a somatic event in AML (Lensch et al. 2003). One of the shortcomings of the present study is that, we could not look for mutation of various group of Fanconi Anemia (A-G) in our patient who showed increased MMC induced chromosome breakage. The FA gene(s) mutation may be inducing non-hereditary, acquired chromosomal breakage in MDS. In FA, the patients exhibit high frequency of chromosome breakage and this instability due to FA gene mutations , develops in to complex clonal chromosomal abnormalities when FA transform to AML or MDS . The same mechanism may be occurring some group of MDS patients as they show high frequency of chromosomal instability (Yunis et al., 1988). However molecular screening of FA gene mutations is essential to correlate the acquired chromosomal damage.

Aknowledgments

We thank Mr.M.V.Zodge and Mr.Anup Jadhav for their technical assistance. Thanks also due to hematologists, Dept of Hematology, K.E.M hospital, Mumbai,India. This work was supported by a core grant to the Institute of Immunohaematology, Indian Council of Medical Research, and Government of India.

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