The myelodysplastic syndromes (MDSs) are a heterogenous group of clonal haematopoietic stem cell disorders that cause one or more peripheral cytopenias due to ineffective haematopoiesis\(^1\). MDS are associated with a high risk of progression to acute leukaemia and with an overall short survival, death being generally due to the consequences of cytopenias or progression to acute leukaemia\(^2\). A succession of MDS classification systems have been developed to facilitate prediction of the risk of progression to acute myeloid leukaemia (AML) and overall survival. The first of these was the French-American-British (FAB) system based on cytomorphologic abnormalities and blast percentage\(^3\). Recently, the World Health Organization (WHO) classification\(^4\) proposed the following groups: RA (refractory anaemia), RARS (refractory anaemia with ringed sideroblast), RCMD (refractory cytopenia with multilineage dysplasia), RAEB (refractory anaemia

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Cytogenetic profile of Indian patients with de novo myelodysplastic syndromes

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**Background & objectives:** Myelodysplastic syndrome (MDS) is a clonal haematopoietic stem cell disorder characterized by ineffective haematopoiesis and leukaemia progression. Cytogenetic analysis has proven to be a mandatory part of the diagnosis of MDS as well as a major indicator for predicting clinical course and outcome. Studies on cytogenetics of MDS are reported mostly from the West and only a few are available from Asian countries. We report herein cytogenetic studies on 40 Indian patients with primary MDS to find out the occurrence and type of chromosome abnormalities and recurring defects.

**Methods:** Cytogenetic analysis was done using GTG banding and karyotyped according to the International System for Human Cytogenetic Nomenclature (ISCN).

**Results:** Of the 40 patients, 19 patients (47.5%) showed clonal karyotypic abnormalities with distribution as follows: 3 of 15 (20%) of refractory anaemia (RA), 4 of 7 (57%) of refractory anaemia with excess blasts-1 (RAEB-1), 4 of 6 (67%) of refractory anaemia with excess blasts 2 (RAEB-2), 2 of 3 (67%) of refractory anaemia with ring sideroblasts (RARS), 2 of 4 (50%) of refractory cytopenia with multilineage dysplasia (RCMD), none (0%) RCMD-ringed sideroblasts (RCMD-RS) and 4 patients with 5q syndrome. The frequent abnormalities observed in our study were -7, 5q-and trisomy 8.

**Interpretation & conclusions:** Two rare chromosomal abnormalities (6q-, 3q-) were found with unknown prognostic significance. Hence, cytogenetic analysis may be incorporated in the routine diagnosis of MDS since there are racial differences in clinical pictures and the molecular events.

**Key words** AML - chromosomal abnormalities - cytogenetics - myelodysplastic syndromes

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The myelodysplastic syndromes (MDSs) are a heterogenous group of clonal haematopoietic stem cell disorders that cause one or more peripheral cytopenias due to ineffective haematopoiesis\(^1\). MDS are associated with a high risk of progression to acute leukaemia and with an overall short survival, death being generally due to the consequences of cytopenias or progression to acute leukaemia\(^2\). A succession of MDS classification systems have been developed to facilitate prediction of the risk of progression to acute myeloid leukaemia (AML) and overall survival. The first of these was the French-American-British (FAB) system based on cytomorphologic abnormalities and blast percentage\(^3\). Recently, the World Health Organization (WHO) classification\(^4\) proposed the following groups: RA (refractory anaemia), RARS (refractory anaemia with ringed sideroblast), RCMD (refractory cytopenia with multilineage dysplasia), RAEB (refractory anaemia
with excess blasts), MDS - U (MDS unclassifiable), and MDS associated with isolated del(5q) chromosome abnormality. Two categories are recognized within the RAEB group: RAEB-1 defined by 5-9 per cent blasts in the bone marrow and <5 per cent blasts in the blood, and RAEB-2, defined by 10-19 per cent blasts in the bone marrow. The WHO classification is more stringent than the FAB classification regarding the percentage of blasts. Chromosomal abnormalities are detected in approximately 50 per cent of patients with de novo MDS and up to 80 per cent in patients with MDS secondary to chemotherapy or other toxic agents. The clonal chromosomal changes are of both numerical and structural types. The most frequently occurring single abnormalities include mainly -5/del (5q), -7/del (7q), del (11q), del (12p)/ (12q), -y and +8. It is observed that the MDS patients from India are younger than those reported from the West and have severe clinical presentation. The response to treatment in these is poorer than the western patients. In view of this, we did conventional cytogenetics in MDS patients attending a tertiary care hospital in New Delhi, India, to find out the occurrence and types of chromosome abnormalities as also recurring defects.

**Material & Methods**

**Patients:** Cytogenetic analysis was performed on 70 randomly selected de novo MDS patients including both new and old cases, presenting to the OPD of Department of Hematology, All India Institute of Medical Sciences (AIIMS), New Delhi, between January 2006 to December 2007. Of the 70 samples, 40 were successfully karyotyped. Follow up data were available for 11 patients. Samples from the old cases were taken when they visited the OPD for follow up. Patients with an ambiguous diagnosis of MDS, those who had previously received chemo/radiotherapy, and those with MDS secondary to a previous malignancy were excluded from the analysis. Patients were treated with lenalidomide or cyclosporine and supportive care. Informed consent was obtained from all patients for the collection of bone marrow (BM) and peripheral blood (PB) samples. The ethical committee of the institute approved the study protocol.

**Methods:** From each patient 0.2-0.5 ml bone marrow or 1 x 10⁶ cells were taken in heparin and cultured in RPMI 1640 (Gibco, USA) supplemented with 20 per cent foetal calf serum (FCS), 2 mM L-glutamine, penicillin and streptomycin (100U/ml) for 72 and 24 h respectively. Colcemid (Gibco) was added two hours before harvesting at a final concentration of 0.1μg/ml. The cells were then treated with hypotonic KCl (Sigma, USA) (0.075 M) for 12-15 min and fixed with methanol/acetic acid (Merck) (3:1 v/v). Cell suspension was dropped on clean chilled slides and flame dried. Gimsa Trypsin Gimsa (GTG) banding was performed by prior trypsinization and subsequent staining with Gimsa and karyotyped according to the International System for Human Cytogenetic Nomenclature (ISCN). At least 20 metaphases were analyzed to demonstrate the clonal nature of the aberrations.

**Results**

**Patients characteristics:** The median age of patients was 42 yr (range 14-75 yr M:F: 1:1.2). Twenty six patients (65%) were under 46 yr and five patients were below 20 yr. Using FAB criteria, 24 (60%) were classified as having refractory anaemia (RA). The other FAB subgroups were as follows: RA with ringed sideroblasts (RARS), 3 (7.5%); RA with excess blasts 11 (27.5%); RA with excess blasts in transformation 2 (5%); chronic myelomonocytic leukaemia 0 (0%). According to WHO criteria, 15 (37.5%) had RA, 3 (7.5%) had RARS, 4 (10%) had 5q- syndrome, and refractory cytopenia with multilineage dysplasia (RCMD), 1 (2.5%) had RCMD-RS, 7 (17.5%) had RAEB-1, and 6 (15%) had RAEB-II (Table I).

**Cytogenetics:** Of the 40 patients karyotyped, 21 (52.5%) had normal cytogenetics and 19 patients (47.5%) had chromosomal abnormalities. The distribution was as follows: 3 of 15 (20%) of RA, 4 of 7 (57%) of RAEB-1, 6 of 6 (100%) of RAEB-2, 2 of 3 (67%) of RARS, 2 of 4 (50%) of RCMD and 0 of 1 (0%) RCMD-RS (Table II). Detailed karyotypes are given in Table III.

Monosomy 7 was the most frequent cytogenetic abnormality detected in 6 of 19 (32%) patients, followed by deletion 5q -detected in four (21%) and trisomy 8 in three (16%) patients; deletion 3q and deletion 6q were observed in two (11%) of cases. Other abnormalities in each case included isochromosome 9q and complex karyotype.

**Transformation to AML:** Of the 40 patients studied, two (5%) progressed to AML. The first patient who had RAEB-I, was diagnosed as MDS with monosomy 7 in September 2006 and transformed to AML-M2 in March 2007. The other patient diagnosed as RAEB-II with trisomy 8 transformed to acute leukaemia within 10 months. Of the patients with normal karyotype, none transformed to acute leukaemia, but one patient...
Table I. Baseline characteristics of patients with MDS

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Median</th>
<th>Min</th>
<th>Max</th>
<th>Mean ±SD</th>
<th>Interquartile range (Q1,Q3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients, no. (n)</td>
<td>40</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (yr)</td>
<td>42</td>
<td>14</td>
<td>75</td>
<td>41.57 ± 16.26</td>
<td>23 (29,52)</td>
</tr>
<tr>
<td>Haemoglobin, g/dl</td>
<td>6.8</td>
<td>3.3</td>
<td>16.1</td>
<td>6.84 ± 2.521</td>
<td>2.7 (5.7,7.7)</td>
</tr>
<tr>
<td>TLC, x10^9/l</td>
<td>4.05</td>
<td>0.8</td>
<td>18.8</td>
<td>4.45 ± 3.117</td>
<td>3.5 (2.5,6)</td>
</tr>
<tr>
<td>Platelets, x10^9/l</td>
<td>100.5</td>
<td>5</td>
<td>194</td>
<td>84.55 ± 52.0</td>
<td>82 (30,112)</td>
</tr>
</tbody>
</table>

FAB classification (n=40), n (%):  
- RA: 24 (60)
- RARS: 3 (7.5)
- RAEB: 11 (27.5)
- RAEB-t: 2 (5)
- CMML: 0 (0)

WHO classification (n=40), n (%):  
- RA: 5q-syndrome 4 (10)
- RARS: RA 15 (37.5)
- RAEB: RARS 3 (7.5)
- RAEB-t: RCMD 4 (10)
- CMML: RCMD-RS 1 (2.5)
- RAEB-I: RAEB-I 7 (17.5)
- RAEB-II: RAEB-II 6 (15)

Transform to AL, no. (%):  
- RA: 2 (5)
- RARS: 2 (67)
- RAEB-I: 1 progress to RCMD
- RAEB-II: 1

Abbreviations as given in foot note of Table I; *Progressed to acute leukaemia

Discussion

Cytogenetic abnormalities are the major determinants in pathogenesis, diagnosis, and prognosis, thus, form the basis for the selection of drugs in individual patients with MDS. MDS show
a characteristic genetic profile with an overweighing of unbalanced abnormalities. The incidence of clonal defects ranges between 32-76 per cent.

In our study, the mean number of metaphases analysed was 20, which is consistent with the criteria for high-quality cytogenetics studies in MDS. By contrast, the frequencies of chromosomal abnormalities previously reported by others were often based on analyses with fewer (>10) metaphases thus, increasing the probability of missing an anomaly.

Studies on MDS are mostly reported from Japan in a large number of patients, with the exception of a few studies from other countries. In Europe, MDS is a disease of the elderly and the median age of patients at the time of diagnosis varies between 65 and 74 yr. In our study the median age of patients at diagnosis was 42 yr which is in concordance with reports from Southeast Asia, Turkey and Central Africa. It has been reported that MDS patients in these areas are generally younger than those from Western countries, and the reason for this difference is not clear. However, it is speculated that the younger age of Asian patients may be due to differential action of various environmental factors, including an increase in exposure to aetiologically relevant risk factors such as organic solvents, pesticides, radiation and environmental pollution.

The incidence of clonal abnormalities in MDS varies between 23-78 per cent. Cytogenetic abnormalities in MDS are associated with a strong prognostic value and in the pathogenesis of the disease. The frequency of clonal cytogenetic abnormalities was 47.5 per cent among patients with primary MDS in our study which is in agreement with published literature. The frequency in the low risk group (RA, RARS and 5q syndrome) was 41 per cent and in high risk group (RAEB1, RAEB2 and RCMD) 55 per cent in concordance to that reported in the literature. In the present study, the most common chromosomal abnormalities were of -7,del(5q),+8,del(6q) and del (3q) in the decreasing order of preponderance. It was similar to the most of the Western studies except for the del (6q) and del (3q) which were not reported as a common chromosomal abnormalities in MDS.

The chromosome status at diagnosis and during the course of the disease is a major prognostic indicator for survival, leukaemic transformation and response to treatment. In most studies, patients with a normal karyotype survived longer and presented leukaemic transformation less frequently than those with an abnormal karyotype. However, in the present study, one patient with RARS having normal kayotype died seven months after diagnosis. Another
patient with RA with normal karyotype reported at the time of presentation as well as during follow up transformed to RCMD. While karyotypic evolution frequently represents a poor risk feature, there may be some cases in which it does not correlate with disease acceleration.\(^\text{23}\)

Trisomy 8 as a single defect has been generally considered as an intermediate or high risk chromosome abnormality.\(^\text{24}\) In our study, one patient with RAEB-II with trisomy 8 transformed to acute leukaemia within 10 months from the time of diagnosis. More studies are needed to accurately evaluate the prognostic significance of karyotype transformation and to show whether specific additional defects may define different prognostic subsets of patients.

The distribution of specific chromosomal abnormalities characterized in the subtypes of leukaemia varies between Western and Asian countries.\(^\text{25}\) Geographical and ethnic differences in the frequency of specific chromosomal aberrations have also been reported.\(^\text{26}\) The incidence of all chromosomal abnormalities has been reported to vary between 37 and 88 per cent in the Indian population and 37 and 50 per cent in China, Thailand, Japan and other European and Western countries.\(^\text{9,10,13,16,17}\)

While the pathogenesis of MDS is still poorly understood, environmental, biological and occupational factors could induce mechanisms that are associated with diverse karyotypes and variable frequency of chromosomal abnormalities.\(^\text{27,28}\) Thus, genetic load and degree of exposure to aetiological agents in various countries owing to socio-economic standards may explain the differences in the incidence and of non-random chromosomal abnormalities among MDS patients in different geographical locations. Taken together, these factors may have a significant contribution in the pathogenesis and variability in the demographic and cytogenetic profile of MDS patients all over the world.

In conclusion, our findings provided an overview of the cytogenetic profile of MDS from India and identified previously undescribed prognostically relevant cytogenetic abnormalities. Presently, cytogenetic investigations in India are performed in only a few hospitals. Prospective studies on a large number of patients help elucidate more precisely the demographic and ethnic differences in the pathogenesis of MDS from different parts of the world.

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References


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