Cytogenetic study of myelodysplastic syndrome from India

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Background & objectives: Myelodysplastic syndrome (MDS) represents a group of clonal haematological disorders characterized by progressive cytopenia reflecting defects in erythroid, myeloid and megakaryocytic maturation. The incidence of MDS is more in older age groups and frequent chromosome abnormalities reported to be monosomies 5 and 7. However, the data on cytogenetic changes in Indian MDS patients are scanty. The present study was therefore undertaken to study the aetiology and frequency of chromosomal changes in MDS patients, attending a tertiary care hospital in Maharashtra, India.

Methods: The study was carried out in 145 MDS patients for six years (2001-2006) at National Institute of Immunohaematology (ICMR), and KEM Hospital, Mumbai, India. The patients were diagnosed according to FAB and WHO classification. Cytogenetic study was carried out using GTG-banding and fluorescence in situ hybridization (FISH) methods. Statistical analysis was done with \( \chi^2 \) and Fisher’s exact test.

Results: Chromosomal abnormalities, including novel chromosome aberrations were detected in 54.48 per cent MDS patients and frequency of chromosomal aberrations increased with increase in age (≥30 yr). Among occupational exposure factors, chromosomal aberrations significantly (\( P<0.05 \)) associated with pesticides exposure.

Interpretation & conclusion: Our findings showed 54.48 per cent chromosome abnormalities including novel chromosome aberrations in MDS patients and these chromosome aberrations were increased with advancing age. In our series a high frequency of younger population (53%) developed MDS, a detailed molecular genetics and aetiological factors need to be studied.

Key words Clonal chromosome abnormality - cytogenetics - myelodysplastic syndrome - occupational exposure - refractory anaemia

Myelodysplastic syndrome (MDS) represents a group of clonal haematological disorders characterized by progressive cytopenia reflecting defects in erythroid, myeloid and megakaryocytic maturation\(^1\). MDS in western countries is mainly found in the elderly population and rarely in the paediatric age group\(^2\). Incidence of MDS is not known in India. Cytogenetic study plays an important role in the diagnosis of primary and secondary MDS. Clonal non random chromosomal changes ranging between 23 and 78 per cent have been reported in MDS patients from United States, Japan and Europe\(^3,5\). However, only a couple of studies are reported from India\(^6,7\). Yunis et al\(^8\) 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, deletions 5q, 7q, 9q and 20q. Although a large number of reports on the risk factors of leukaemia have been published, little is known about the risk factors of MDS. The occupational exposure to chemicals, radiations and habits such as smoking, alcohol consumption are reported to be associated with the development of MDS. The data on cytogenetics in Indian patients with MDS are extremely sparse and have not been correlated with occupation of the patients. The present study was carried out to elaborate the range of cytogenetic anomalies found in Indian patients with MDS, and their correlation with occupational data.

**Material & Methods**

Study was carried out from 160 (108 males, 52 females) adult patients suspected to have primary MDS attending the out patients of Department of Haematology, KEM Hospital, Mumbai, India, for the diagnosis and treatment during January 2001-2006. The age group ranging from 19 to above 60 yr and mean age was calculated as 44.3 ± 2.6 yr.

The Institutional ethical committee of the National Institute of Immunohaematology, KEM hospital, Mumbai, India, approved the study protocol. Informed consent was obtained prior to the collection of the bone marrow (BM), blood, and epidemiological data. Patients with secondary MDS (therapy-induced MDS) were excluded. All patients completed a pre-validated questionnaire available in both English, Hindi and local language that assessed occupational exposure.

The BM (2 ml) or blood (4 ml) samples collected in F-10 nutrient media (Sigma, USA) with 20 per cent foetal bovine serum (Sigma, USA) were arrested with colcemid (Sigma, USA) using direct and 24 h culture methods. The cultures treated with 0.075 M hypotonic solution (KCl) were fixed with methanol: acetic acid (3:1v/v). The chromosomal preparations obtained by dropping 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 according to International System for Human Cytogenetic Nomenclature (ISCN) 2005. Fluorescence in-situ hybridization (FISH) analysis was carried out using centromeric probes 5, 7, and 8 (Abott, Germany) and locus specific probes 5q33, 7q31-33 (Abott, Germany) according to standard procedure.

**Statistical analysis**: Contingency table data were analyzed using χ² analysis. Fisher’s exact test was used for the data with small expected frequencies.

**Results**

Cytogenetic study was successfully carried out in 145 out of 160 MDS patients. The karyotyping could not be done in 15 patients, due to hypocellular BM or diluted BM or inadequate sample. The age of patients ranged from 5 months to 75 years with a mean age 44.34 yr (SD ± 2.6). The distribution of MDS subgroups and age-wise distribution of such patients is presented in Table I. A high frequency (53.8%) of MDS was observed in patients <45 years age. A high frequency of MDS subgroup in our series were refractory anaemia (RA) (37.93%), refractory anaemia with excess blasts (RAEB) (28.27%), and refractory anaemia with excess blasts in transformation (RAEB-t) (20.69%). Of the 145 patients studied with the combination of conventional cytogenetics and FISH, 79 (54.48%) had clonal chromosomal abnormality (Table II). A high frequency (19%) of deletions was detected compared to other chromosome aberrations such as monosomics, trisomics and translocations (12% each). Of the 79 patients with chromosomal aberrations, 10 (12.66%) were found to had novel chromosomal abnormalities including, t(9;12)(q12;q24.3), t(1;2)(q13;q12), t(11;14) (q13;q32) in refractory anaemia (RA), dic(1;16)(q21;p13.3) t(1;22),t(9;12;22)(q34;q15;q11),

### Table I. Distribution of subtypes of MDS by age

<table>
<thead>
<tr>
<th>Subtype</th>
<th>No. (%)</th>
<th>&lt;19</th>
<th>20-24</th>
<th>25-29</th>
<th>30-34</th>
<th>35-39</th>
<th>40-44</th>
<th>45-49</th>
<th>50-54</th>
<th>55-59</th>
<th>&gt;60</th>
</tr>
</thead>
<tbody>
<tr>
<td>RA</td>
<td>55(37.93)</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>6</td>
<td>8</td>
<td>10</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>RAEB</td>
<td>41(28.27)</td>
<td>-</td>
<td>2</td>
<td>2</td>
<td>5</td>
<td>5</td>
<td>8</td>
<td>3</td>
<td>4</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>RAEB-t</td>
<td>30(20.69)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>3</td>
<td>5</td>
<td>3</td>
<td>4</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>CMMOL</td>
<td>10(6.90)</td>
<td>-</td>
<td>-</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>RARS</td>
<td>9(6.21)</td>
<td>-</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>145</td>
<td>2</td>
<td>5</td>
<td>11</td>
<td>19</td>
<td>18</td>
<td>23</td>
<td>13</td>
<td>15</td>
<td>18</td>
<td>21</td>
</tr>
</tbody>
</table>

RA, refractory anaemia; RAEB, refractory anaemia with excess blasts; RAEB-t, refractory anaemia with excess blasts in transformation; CMMOL, chronic myelomonocytic leukaemia; RARS, refractory anaemia with ring sederoblasts
t(10;14)(q31;q21) in refractory anaemia with excessive blasts transformation (RAEB). der(4)t(1;4)(q21;q35) +mar, t(7;13), t(8;21) (q22;q22), t(1;X)(p21;q31), -7 in refractory anaemia with excessive blasts transformation (RAEB-t), del(2)(q33-ter) in chronic myelomonocytic leukemia (CMMOL) and trisomy 14 in refractory anaemia with ring sideroblasts (RARS).

A significantly (P<0.05) high frequency (58.27%) of chromosomal aberrations was detected in ≥ 30 years age group MDS patients compared to < 29 yr (27.78%) (Table III). Among 145 MDS patients studied, 59 (40.69%) were found to be exposed to chemical mutagens such as pesticides, benzene, asbestos, arsenic etc. The duration of exposure was calculated above and below 15 yr exposure to the occupational mutagens. The chromosomal aberrations were detected in ≥ 15 yr occupational exposure group. There was no significant difference between chromosomal aberrations and normal karyotypes of patients exposed to benzene, asbestos, and arsenic (Table IV).

**Discussion**

The MDS are clonal disorders of haematopoietic stem cells characterized by ineffective haematopoiesis leading to blood cytopenias and by high incidence of
progression to acute myeloid leukemia (AML)\textsuperscript{18}. These disorders generally arise de novo, but may occur years after exposure to mutagenic chemotherapy\textsuperscript{11}. The precise incidence of de novo MDS is not known. In one study annual incidence per 100,000 population was estimated as 0.5 for people less than 50 yr of age compared to 5.3 for 50 to 59, 15 for age 60 to 69, 49 for 70 and 79 to 89 for ages older than 80\textsuperscript{19}. Aul et al\textsuperscript{20} reported a crude annual incidence of 4.1/100,000 for MDS. The mean age was approximately 65 yr. In our study MDS was seen in younger population as the mean age was 44.34 yr. Also there was a higher incidence (53.79\%) in the age group \( \leq 19-44 \) yr. The reason for the higher frequency of MDS in the young Indian population remains to be studied further. However, high incidence of MDS in younger population was also reported from Japan\textsuperscript{21}. Clonal chromosome changes in the BM cells are known to vary between 23 and 78 per cent\textsuperscript{1-5}. In our study, about 55 per cent patients showed clonal chromosomal abnormalities, some of which are known to be typically associated with MDS/AML and some were distinctly unusual. The conventional cytogenetics revealed 37 per cent chromosome aberration and FISH had an advantage in detection of another 17 per cent chromosome aberrations mostly monosomies 5 and 7, trisomy 8, deletions 5q33, 7q31. Chromosomal aberration frequency ranging 50-80 per cent have been reported in studies using FISH and conventional cytogenetics\textsuperscript{22,23}. The two reported studies from India showed 37.5 and 88 per cent chromosome anomalies including frequent chromosomal abnormalities like deletion 5,7,11 monosomy 5 and trisomy 8\textsuperscript{6,7}, and also presented new chromosomal abnormalities such as i(17q) inv(5) del (17), del (8q) which are less frequently reported from western population. In our study, these abnormalities were not detected and some new chromosomal anomalies were detected. A significantly increased frequency of chromosome aberrations detected in above 30 yr age group suggests the genetic changes occurring with advancing age in Indian patients and also supports the previous reports of increasing chromosomal aberrations in advanced age group. However, as novel chromosomal abnormalities were detected in our study, Indian patients may have different underlying aetiological reason for the development of MDS.

Aetiological factors of the MDS are largely unknown, with the exception of alkylating agents, ionizing radiation and benzene\textsuperscript{24-26}. Certain other risk factors (solvents, ammonia, exhaust gases, metals, pesticides, alcohol) have been suggested\textsuperscript{27}. In our study, occupational factors such as exposure to pesticides, semi metals, metals, inorganic fumes/solvents were identified as a risk factors for the development of MDS. In India chemical fertilizers and pesticides are used in agriculture. The pesticide induced chromosomal abnormalities have been reported in MDS\textsuperscript{28}. Though chromosomal aberrations were not significant in our patients exposed to organic chemicals (benzene), inorganic dusts (asbestos), and metals (copper), these compounds have been reported to be mutagenic. Recently a high frequency of chromosomal breakage was reported in MDS patients and Fanconi anaemia pathway presumed to be alter the DNA repair mechanism and leads to chromosomal instability\textsuperscript{29,30}.

The chromosomal abnormalities frequency was improved with the combination of GTG-banding FISH techniques and FISH has an advantage in detection of detecting aneuploidy and deletions of common chromosomal aberrations. The Indian MDS patients may have different underlying aetiological factors as significant number of patients had novel chromosomal aberrations. Though environmental factors especially pesticides has a role in chromosomal changes and developing MDS, there is a need to study molecular genetic and epigenetic factors in younger age group of MDS patients to understand the nature of the disease.

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