INTRODUCTION

Myelodysplastic syndromes (MDS) represent a heterogeneous group of haematological disorders characterized by ineffective hematopoiesis and an increased risk of developing acute myelogenous leukemia (AML). The classification of myelodysplastic syndromes is based on the morphological criteria proposed by the French-American-British (FAB) and World Health Organization (WHO) groups. Accurate enumeration of blast cells is essential for diagnosis of myelodysplastic syndrome and for assignment to prognostic groups. The new WHO classification system takes into account the medullary and peripheral blast count as well as the degree of dysplasia in the different cell lines. Accurate diagnosis and classification are essential for subgroup identification and prognostic assessment of patients with MDS. The separation of MDS from acute leukemias has been redefined by WHO using a cutoff value of 20% peripheral and/or medullary blasts and introduction of deletion 5q (del 5q) entity which is characterized by special morphologic and hematologic features. The classification of myelodysplastic syndrome is a continuously evolving process. The WHO revised classification of MDS divides the syndrome into refractory cytopenia with unilineage dysplasia (RCUD) refractory anaemia (RA), refractory neutropenia (RN), refractory thrombocytopenia (RT) refractory anaemia with ring sideroblasts (RARS) refractory cytopenia with multi lineage dysplasia (RCMD) myelodysplastic syndrome unclassified (MDS-U) deletion 5q-syndrome, refractory anaemia with excess of blasts I (RAEB I) and refractory anaemia with excess of blasts II (RAEB II).

The incidence of MDS is about 5 per 100,000 persons per year in the general population, but it increases to 20 to 50 per 100,000 persons per year after 60 years of age. In children and adolescents, MDS are uncommon disorders, accounting for less than 5% of hematopoietic malignancies, with great heterogeneity in presentation and clinical course. In the paediatric haematological malignancies, internationally, MDS represents an annual incidence of 0.5 to 4 per million. In Pakistan, a few epidemiological studies that were carried out showed male preponderance, a younger age of onset and relatively higher frequency of aggressive disease as compared to studies from America and Europe. A single centered study in Pakistan reported MDS to be 4.6% of all haematological malignancies in patients under 15 years of age.
This study was conducted to analyze the WHO system of classification of MDS, to assess the applicability of WHO classification on a cohort of Pakistani MDS patients, to elaborate the epidemiological and clinico-pathological features of MDS patients in Pakistan and compare it with other regional and international reports.

**METHODOLOGY**

This observational study was carried out at the Department of Haematology, Shaikh Zayed Hospital (SZH), Lahore, from April 2004 to March 2006. All the patients visiting Shaikh Zayed Medical Complex during the study period were included in the study by non-probability purposive sampling. Forty six patients were found to fulfill the WHO criteria for MDS during this time period. Patients who were suffering from deficiencies of iron, vitamin B<sub>12</sub> and folic acid were excluded. Also excluded were those cases diagnosed with infections i.e. tuberculosis, leprosy, typhoid, chronic active hepatitis, AIDS and chronic inflammations i.e. rheumatoid arthritis and systemic lupus erythematosus. A detailed history was taken about fever; weight loss (about 10% in the last 6 months); bleeding from any site including multiple bruises with minor trauma, purpura, epistaxis, gum bleed, hematemesis, haemoptysis, haematochezia, malaena, haematuria, manorrhagia, excessive bleeding from wounds or cuts or after a surgical procedure; melaena, haematuria, manorrhagia, excessive bleeding into the skin (e.g. bruises and purpura), signs of bleeding from the nose, or alimentary canal, vagina or anal canal and accessible lymphadenopathy in the cervical, axillary and inguinal region. Hepatomegaly and splenomegaly were sought in abdominal examination and confirmed by abdominal ultrasound.

The blood sample from the patients were tested in Sysmex KX 21 for complete blood counts (CBC) including haemoglobin (Hb), total leucocyte count (TLC) and platelet count and mean cell volume (MCV). Blood film was stained by May-Grunwald-Giemsa stain and peripheral smear examination was carried out. Bone marrow aspirates were done from right posterior iliac crest. May-Grunwald-Giemsa staining was performed on the aspirate which was then examined for cellularity, evidence of dysplasia, erythropoiesis, myelopoiesis and megakaryopoiesis. Cellularity assessment was based on visual examination and graded into three groups; normocellular (30-50% of intertrabecular spaces occupied by haematopoietic cells), hypercellular (> 50%), hypocellular (< 30%). Blast percentage of nonerythroid nucleated bone marrow cells was calculated from a myelogram of 500 cells. The blast cells were confirmed as myeloid blasts by staining with myeloperoxidase. Non specific esterase staining was done for confirming blasts for monocytic lineage. Perl staining was done on patient’s bone marrow aspirate slides for ring sideroblasts. Bone marrow trephine biopsies were stained by haematoxylin and eosin. The slides were examined for cellularity divided into 3 grades i.e. “increased, normal, decreased”; reticulosis and presence of abnormal localization of immature precursors (ALIP). Silver impregnation was carried out to grade reticulosis from 0 to 4 depending on severity. The data was used to classify patients of MDS.

The collected data was entered into SPSS version 10.0 for analysis. Nominal data of variables including pallor, fever, bleeding, weight loss, splenomegaly, hepatomegaly, lymphadenopathy and bone marrow features of cellularity, erythropoiesis, myelopoiesis, megakaryopoiesis and ALIP were recorded as frequency/percentages. The cohort was divided into 2 groups on the basis of age above and below 14 years. The mean age of the paediatric group and the adult group was calculated separately. The variables in CBC including haemoglobin (Hb), TLC, platelet count and mean cell volume were recorded as mean ± standard deviation for each group.

**RESULTS**

Forty six patients of primary MDS were included in the study. There were 28 males and 18 females. Four were children under 14 years of age and 42 were adults. The cohort was classified according to WHO criteria of classifying MDS. There were 12 cases of RCUD-RA, 24 cases of RCMD, 1 case of RCMD-RS, 3 cases of MDS unclassified, 3 cases of RAEB-I and 3 cases of RAEB-II identified in the study group.

The mean age of the entire cohort classified in MDS by WHO criteria was 43.09 years. For adult cases, the mean age came to 46.21 years. The mean age for RA was 43.08 years, for RCMD was 43 years, for RARS 50 years, for MDSU 44.66 years, for RAEB I was 44 years and for RAEB II was 32.66 years.

The commonest complaint of the patients was pallor and easy fatigability effecting 37 cases. Fever was seen in 22 cases, followed by bleeding reported in 17 cases. Lymphadenopathy (n=8), splenomegaly (n=6), weight loss (n=5) and hepatomegaly (n=3) were infrequent.

The result of complete blood counts are documented in Table I. Anaemia was the most common finding effecting 43 patients (93.4%) with a mean haemoglobin (Hb) of 6.52 g/dl (range of 2.6-14.2 g/dl). Hb was less than 8 g/dl in 37 (80.4%) patients. Only 2 patients had Hb higher than 12 g/dl at presentation. Mean total leucocyte count (TLC) was 3.43 x 10<sup>9</sup>/l (range of 0.7-16.6 x 10<sup>9</sup>/l). Mean platelet count (Plt ct) was 59.63 x 10<sup>9</sup>/l (range of 3-358 x 10<sup>9</sup>/l).

The RAEB-I had a mean age of 44 years, mean Hb of 6.36 g/dl, TLC of 2.63 x 10<sup>9</sup>/l, platelet count of 25 x 10<sup>9</sup>/l.
and mean cell volume (MCV) of 89.4 fl. The RAEB-II had a mean age of 32.6 years, mean Hb of 5.96 g/dl, TLC of 3.6x10^9/l, platelet count of 77x10^9/l and MCV of 93.33 fl. Pancytopenia was the most common feature present in 33 cases. Bicytopenia was seen in 12 patients. The combination of anaemia and thrombocytopenia was seen in 5 patients; anaemia with leucopenia was seen in 3 patients and the combination of thrombocytopenia and leucopenia was seen in 2 patients. Anaemia was the sole presentation in 3 patients. Only 1 patient presented with isolated thrombocytopenia. Peripheral smear revealed dimorphic red cell morphology consisting of either normochromic normocytic RBCs or hypochromic microcytic RBCs mixed with a prominent population of macrocytes.

Bone marrow trephine biopsy was found to be hypercellular in 27, normocellular in 11, and hypocellular in 8 cases. Bone marrow aspirate showed evidence of dyserythropoiesis in atleast one cell line in all patients. Dyserthrypoiesis was present in 41 of aspirates (89.1%). Dysmyelopoiesis was seen in 18 of aspirates and morphologically abnormal megakaryocytes were identified in 26 of the aspirates. Dysmegakaryopoiesis was confirmed in trephine biopsies and micro-megakaryocytes were seen in 50% of the biopsies.

Grade III reticulosis was seen in 7 cases (5 cases of RCMD, 1 case each of RAEB I and RAEB II). Abnormal localization of immature precursors (ALIP) was present in 2 cases of RAEB I, 2 cases of RAEB II, 6 cases of RCMD and 3 cases of RCUD-RA.

### DISCUSSION

MDS has long been considered a disease of the elderly but in Pakistani population it was identified at a younger age. This study consisted of 46 patients aged from 2 years to 81 years with mean age of 43.09 years. This result is similar to reports of Indian population with mean age of 45 years\(^{16}\) and 50 years.\(^{17}\) From China the mean age of adults with MDS was reported as 49 years\(^{18}\) and 58 years.\(^{19}\) This is in wide contrast to studies from Europe which estimate the mean age at diagnosis to be above 70 years.\(^{20}\) Mean age of 69 years was reported from Turkey.\(^{21}\) Lau \textit{et al.} studied Asian population residing in Singapore and reported a mean age of 64 years.\(^{22}\) The studies based on South East Asian population including Pakistan, India, China showed a much younger age of onset as compared to European studies. The results from Turkey, while those of Singapore, Japan, Taiwan, Thailand and Spain were somewhere between those of Asia and Europe.\(^{20-23}\)

The male to female ratio was found to be 1.5:1 in this study which compares well with 1.7:1 reported by Dakshinamurthy \textit{et al.}\(^{17}\) and 1.8:1 reported by Chatterjee \textit{et al.}\(^{16}\) both from India. From Singapore, Lau \textit{et al.}\(^{22}\) reported 1.5:1, from UK, Tefferi reported 1.8:1.\(^{14}\) From Japan, Oguma \textit{et al.}\(^{23}\) reported a ratio of 1.7:1. In WHO classification RCMD, RCMD-RS, RAEB-I, RAEB-II showed a marked male preponderance (male female ratio 1.8:1) while MDS unclassified showed a female predominance with a ratio of 1:2. The relatively aggressive categories consisted of higher proportion of male patients.

Bone marrow hypocellularity was encountered in 6 cases of RCMD and were labeled hypoplastic MDS. One case each of RAEB-I and RAEB-II also showed hypocellularity for age. Hypoplastic MDS was seen commonly in the RCMD subgroup as compared to RAEB. This was also the finding in a Chinese study by Huang \textit{et al.} who reported refractory anemia (RA) to be the predominant sub group in hypoplastic MDS.\(^{25}\) Reticulin was predominantly increased in RCMD and RAEB compared to RA implying predilection for aggressive disease categories.

Table II compares data from other similar studies with the present one. It can be seen that RCMD and RA together constituted the major bulk of the disease in all of the studies under consideration (50-75%). In studies from South East Asia (India, China, Pakistan) the number of RCMD patients was at least double the number of RA cases, suggesting a similar disease epidemiology in the region. RCMD has become a better category Akiba \(^{24}\) Chatterjee\(^{16}\) Wang XQ\(^{19}\) Present study

### Table I: Distribution of haemoglobin, total leucocyte count, platelet count and mean cell volume in 46 cases of MDS classified by WHO classification.

<table>
<thead>
<tr>
<th>WHO diagnosis</th>
<th>No. of cases</th>
<th>Hb</th>
<th>TLC</th>
<th>Pt ct</th>
<th>MCV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean ±SD</td>
<td>mean ±SD</td>
<td>mean ±SD</td>
<td>mean ±SD</td>
<td></td>
</tr>
<tr>
<td>RCUD-RA</td>
<td>12</td>
<td>5.9±2.3</td>
<td>4.7±4.0</td>
<td>78.6±78.8</td>
<td>100.4±90.0</td>
</tr>
<tr>
<td>RCMD</td>
<td>24</td>
<td>6.6±2.6</td>
<td>2.4±1.0</td>
<td>50.9±53.7</td>
<td>95.9±11.9</td>
</tr>
<tr>
<td>RCMD-RS</td>
<td>1</td>
<td>5.1±0.1</td>
<td>0.8±0.1</td>
<td>124±10.0</td>
<td>98.5±1.0</td>
</tr>
<tr>
<td>MDS-I</td>
<td>3</td>
<td>8.9±5.5</td>
<td>7.6±5.8</td>
<td>49±38.0</td>
<td>92.2±2.1</td>
</tr>
<tr>
<td>RAEB-I</td>
<td>3</td>
<td>6.3±3.4</td>
<td>2.6±0.8</td>
<td>25±4.0</td>
<td>89.4±1.9</td>
</tr>
<tr>
<td>RAEB-II</td>
<td>3</td>
<td>5.9±1.8</td>
<td>3.6±1.8</td>
<td>77±35.1</td>
<td>93.4±7.6</td>
</tr>
<tr>
<td>Total</td>
<td>46</td>
<td>6.4±2.6</td>
<td>3.6±2.2</td>
<td>67.4±41.6</td>
<td>94.9±5.6</td>
</tr>
</tbody>
</table>

RCUD-Refactory cytopenia with unilineage dysplasia; RA-Refractory anaemia; RCMD-Refactory cytopenia with multilineage dysplasia; RARS-Refractory anaemia with ring sideroblasts; RCMD-RS- Refractory cytopenia with multilineage dysplasia and ring sideroblasts; RAEB- Refractory anaemia with excess of blasts.

Table II: Comparison of distribution of MDS cases classified by World Health Organization.

<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td>RCUD-RA</td>
<td>33.00</td>
<td>19</td>
<td>2.3</td>
<td>26.1</td>
</tr>
<tr>
<td>RCMD</td>
<td>20.24</td>
<td>44</td>
<td>69.6</td>
<td>52.2</td>
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<tr>
<td>RARS</td>
<td>*</td>
<td>1</td>
<td>1.2</td>
<td>0.0</td>
</tr>
<tr>
<td>MDS-RS</td>
<td>*</td>
<td>4</td>
<td>2.2</td>
<td>6.5</td>
</tr>
<tr>
<td>MDS unclassified</td>
<td>*</td>
<td>4</td>
<td>2.3</td>
<td>6.5</td>
</tr>
<tr>
<td>RAEB-I</td>
<td>11.96</td>
<td>10</td>
<td>6.5</td>
<td>6.5</td>
</tr>
<tr>
<td>RAEB-II</td>
<td>13.8</td>
<td>9</td>
<td>24.1</td>
<td>6.5</td>
</tr>
</tbody>
</table>

RCDU-Refactory cytopenia with unilineage dysplasia; RA-Refractory anaemia; RCMD-Refactory cytopenia with multilineage dysplasia; RARS-Refractory anaemia with ring sideroblasts; RCMD-RS- Refractory cytopenia with multilineage dysplasia and ring sideroblasts; RAEB- Refractory anaemia with excess of blasts.

All figures express percentage of cases. *data not available.

recognized entity over the last few years which may possibly be contributory to its progressively higher reported percentage over the years. RAEB I and II showed the second highest number of cases (12-25%). RARS, RCMD-RS and MDS U were relatively uncommon in all of the mentioned reports.

The limitations of this study included lack of cytogenetic testing due to which del 5q- cases were not identified. The clinical spectrum of RAEB-I and RAEB-II was different, however, lack of follow-up information was a restricting factor on elaborating this point further. It is suggested that larger multicentre studies be carried out which should include cytogenetic evaluation and follow-up to gather additional information of the spectrum of MDS in Pakistan.

CONCLUSION
The WHO classification system served to differentiate several disease subcategories with well-defined clinical and morphological disease patterns. The studied cohort of MDS had a relatively younger age. Anemia was the most common presentation. Erythrocytes were frequently macrocytic. Erythropoiesis in the bone marrow was abnormal with usually megaloblastoid features. Dysplasia in the other two cell lines was not infrequent.

Acknowledgement: The authors are thankful to Dr. Arshad Kamal Butt, Associate Professor, Gastro-enterology, Shaikh Zayed Medical Complex, Lahore, for his assistance in the statistical review of this study.

REFERENCES


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