CHAPTER I

INTRODUCTION
1.13. **Introduction**

Myelodysplastic syndromes (MDS) are clonal disorders characterized by ineffective hematopoiesis and subsequent development into acute myeloid leukemia (AML). The consequences of ineffective hematopoiesis are peripheral cytopenias that frequently involve all three blood cell lineages namely, erythroid, granulocyte, and megakaryocyte. Expansion of an abnormal clone is characterized by morphological dysplasia, impaired differentiation, defective cellular functions, and genetic instability. While, anemia and its related symptoms are the most common clinical manifestations for most MDS patients [1], peripheral blood cytopenia in combination with a hypercellular bone marrow (BM) exhibiting dysplastic changes are the hallmark of MDS [2].

1.14. **Incidence and etiology**

The true incidence and prevalence of MDS are uncertain because cancer registries often fail to capture MDS cases due to technical limitations in early detection. MDS can affect people of any age, including children with an overall incidence of around 1:50,000 [3]. It has also been shown that a risk of developing MDS increases steadily with advancing age, are most likely due to acquisition of DNA damage, depletion of stem cells and/or cumulative exposure of the bone marrow to environmental stress and toxins.

MDS is classified based on etiology as primary (*de novo*) and secondary (treatment related). Initial studies have shown evidence for clonality in all forms of MDS, even in their early stages [4]. Still it is under debate, that whether recurrent cytogenetic abnormalities were considered as the primary cause of disease or they are secondary changes that arise due to cytogenetically undetectable initiating lesions in a clonal hematopoietic stem cell population [5].
Other risk factors for MDS include chemotherapy or radiotherapy, occupational exposures to solvents or agricultural chemicals; however, more than 80% of patients with MDS did not have an obvious history of exposure to any of those agents [6,7]. Furthermore, as it has been reported earlier that risk of developing MDS increases steadily with advancing age and that bone marrow of elderly persons are especially vulnerable; this suggests that the environment in which a patient lives may influences the risk of MDS development.

The risk for MDS is seen increased in certain genetic syndromes such as the Diamond-Blackfan syndrome (pure red-cell hypoplasia with craniofacial, skeletal, or cardiac defects), the Shwachman-Diamond syndrome (neutropenia, exocrine pancreatic insufficiency, short stature), dyskeratosis congenita (anemia and thrombocytopenia with cutaneous pigmentation, nail dystrophy, leukoplakia), Fanconi’s anemia (aplastic anemia with short stature and other skeletal abnormalities), and severe congenital neutropenia [8].

In contrast, there is little information on hereditary predispositions for nonsyndromic forms of the disease, with the exception of a familial platelet disorder associated with a monoallelic germ-line mutation in \textit{RUNX1}, the gene encoding runt-related transcription factor 1 on chromosome 21q22. Mikhail \textit{et al} [9], has reported that the above genomic region is frequently involved in chromosomal translocations and somatic point mutations in the acute leukemias, sporadic MDS, MDS with myeloproliferative features and therapy-related myeloid neoplasms.

1.15. Diagnosis

Diagnosis of MDS is generally based on clinical manifestations and examination of cell morphology either in the peripheral blood and/or bone marrow. However the minimum diagnostic criteria are based on the combinations of all the three which are discussed below:
1.15.1. Clinical symptoms

Generally, diagnosis of MDS is made in two clinical settings. Many patients were present with signs or symptoms suggestive of a blood disorder—especially fatigue, exercise intolerance, pallor, infection, or inappropriate bleeding and bruising. Alternatively, an asymptomatic individual may be found to have blood and bone marrow features that are typical of MDS during a medical evaluation performed for another reason [10].

1.15.2. Peripheral blood findings

The peripheral blood examination of MDS patients showed severe anemia in about >80% of patients with hemoglobin concentrations <10g/dL [11]. The anemia associated with MDS is usually normocytic or macrocytic; microcytic or hypochromic red blood cell indices suggest an acquired or inherited hemoglobinopathy, concomitant iron deficiency, or both. Approximately 40% of patients with MDS are neutropenic at diagnosis, and many more become neutropenic as the disease evolves. Occasionally, neutropenia or thrombocytopenia was seen in the absence of anemia. Acquired functional and morphologic abnormalities of blood cells are common in MDS and frequently exacerbate the consequences of cytopenias. These defects include acquired α-thalassemia with hemoglobin-H in erythrocytes (β-thalassemia, membrane disruption, and enzymopathies), pseudo-Pelger-Huet and hypogranular neutrophil morphology [Figures: 1.1 A and B] with impaired chemotaxis and microbicidal activity, dysfunctional platelet activation and aggregation with storage pool deficiency or low surface glycoprotein expression [12-14].

The presence of unexplained monocytosis, peripheral blood cytopenia(s), erythrocyte macrocytosis (even if the hemoglobin level is normal), or cellular atypia and dysplasia on a peripheral blood test should prompt further evaluation. Although not specific for MDS, the combination of all these features is extremely suggestive of the confirmation. In addition to the
characteristic findings, the presence of circulating undifferentiated blasts indicates advanced stage of MDS or progression to acute leukemia. Patients with an MDS-associated feature should include bone marrow aspirate and biopsy, once easily remediable causes for the blood findings have been excluded.

1.15.3. Differential diagnosis

On the other hand, in MDS the macrocytes are usually oval shaped as a consequence of megaloblastoid red cell maturation; exclusively round macrocytes are more consistent with endocrinopathy, disordered cholesterol metabolism, or liver disease, and bone marrow examination is not immediately necessary if these alternative diagnoses are confirmed. Other MDS look-alikes diagnosable by medical history, physical examination, and simple blood tests include cyanocobalamin (vitamin B12) or folate and copper deficiency, human immunodeficiency virus (HIV) infection, or exposure to a drug that causes megaloblastoid erythropoiesis (eg, valproic acid, zidovudine, hydroxyurea, folate antagonists). Also, individuals undergoing routine follow-up evaluations for a hematologic disorder such as lymphoma or a plasma cell dyscrasia also found to have MDS [15-21]. Sometimes a form of MDS was discovered incidentally at diagnosis or staging of another malignancy and before beginning therapy [22,23].

1.15.4. Bone marrow findings

A good quality core biopsy of bone marrow can be invaluable in cases of suspected MDS, allowing determination of overall bone marrow cellularity and tissue architecture [24,25]. Thus, a well-stained bone marrow aspirate is the best tool to assess hematopoietic cellular morphology, determine the extent of dysplasia, atypia and to measure the proportion of bone marrow cells that are undifferentiated myeloblasts [26].
The bone marrow usually has normal or increased cellularity, and cytopenia in the peripheral blood is indication for ineffective hematopoiesis. Morphologic abnormalities include megaloblastic red-cell precursors with multiple nuclei or asynchronous maturation of the nucleus and the cytoplasm. Dyserythropoiesis (Figure: 1.1C), ringed sideroblasts (Figure: 1.1D), erythroid precursors with iron-laden mitochondria, were although occasionally identified, they also be seen in unrelated benign and malignant hematologic conditions. There is often a predominance of immature myeloid cells, and granulocytic precursors may show asynchronous maturation of the nucleus and the cytoplasm [27]. Mature granulocytes are often hypogranular and hypolobulated (Figure: 1.1F) (the pseudo-Pelger-Huët anomaly). Megakaryocytes may also have few nuclear lobes and are often small (micromegakaryocytes) (Figure: 1.1E).

Dysplastic abnormalities in all lineages can include nuclear and cytoplasmic blebs, karyorrhexis, and misshapen nuclei. At more advanced stages of myelodysplasia, the number of myeloblasts was shown to be increased. However, whenever the dysplastic abnormalities in bone marrow fails to establish a diagnosis of myelodysplasia [28], other causes should be ruled out by a careful history taking, physical examination, and laboratory evaluation.

Core biopsy may reveal features that suggest an alternative or additional diagnosis, including features that are uncommon in MDS, such as extensive reticulin deposits, collagen fibrosis or megakaryocyte clustering, all of which are associated more commonly with a myeloproliferative disorder than with MDS and are generally detectable only on bone marrow biopsy [26,29]. The typical megakaryocyte atypia that is associated with the 5q– syndrome is also easily recognized in biopsy findings. Small, early myeloid precursor cells often cluster together in MDS and may localize abnormally away from the bone marrow endosteum, a phenomenon called abnormal localization of immature precursors (ALIP) [30,31].
Figure: 1.1
Morphologic features of peripheral blood and bone marrow in the MDS

A. Peripheral blood with RARS, with dimorphic red cells; some are normochromic and others are hypochromic (arrow). Also, anisocytosis with occasional macroovalocytes (arrowhead).

B. Peripheral blood sample with RAEB, demonstrating pseudo-Pelger–Huet cells with hypercondensed chromatin and hypolobulated nuclei and virtually colorless cytoplasm (arrow).

C. Dyserythropoiesis (arrows) in a bone marrow.

D. Ring sideroblasts (arrows).

E. Dysplastic small megakaryocytes (arrows) with mono or bilobed nuclei and mature granular cytoplasm in RAEB.

F. Medium sized megakaryocytes with hypolobulated nuclei (arrows) in isolated del (5q) case.

Source: [50]
More than 90% of patients with MDS have bone marrow biopsy findings that are either normocellular or inappropriately hypercellular for their age [32]. Excessive apoptosis of bone marrow precursor cells is believed to account for MDS-associated ineffective hematopoiesis and may explain the seeming paradox of hypocellular blood coexisting with hypercellular bone marrow [33-38]. However, the bone marrow in MDS is sometimes hypocellular for patient age, and difficult to distinguish MDS from aplastic anemia on morphologic grounds [39-42]. The presence of a clonal chromosomal abnormality (discussed subsequently) associated with hypocellular bone marrow supports a diagnosis of MDS rather than AA, but the overlap between MDS and AA is substantial, and a rigid distinction may not always be necessary or possible [43].

1.15.5. Minimal diagnostic criteria

The minimal diagnostic criteria to be adopted is summarized and given in Figure: 1.2. The quality of the bone marrow specimen is important [44-46]. Ideally, at least 200 bone marrow cells and 20 megakaryocytes should be examined to be confident about the diagnosis, and at least 10% of these should be dysplastic [47,48]. With use of karyotypic abnormalities and clonal hematopoiesis as gold standards, the blood and bone marrow morphologic findings most specific for MDS include pseudo-Pelger Huet neutrophil morphology, hypogranular or agranular neutrophilic cytoplasm, micromegakaryocytes, ringed sideroblasts, and an increased bone marrow blast proportion (5-20%) as shown in Figure: 1.2 [45,48]. There are cases for which MDS is a possible explanation for cytopenias or other blood features, yet a diagnosis cannot be definitively established, despite careful examination of a well-prepared bone marrow specimen and review of supplemental tests by an experienced hematopathologist [44,49].
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Figure: 1.2
Algorithm for the Classification of Primary Myelodysplastic Syndromes (MDS)

Minimal criteria for MDS diagnosis:
Presence of >10% dysplastic cells in bone marrow within a specific myeloid lineage
Exclusion of AML (>20% peripheral-blood or bone marrow blasts) and CMML (monocyte count of >1×10⁹/L)


Source: [50]
1.15.6. Genetic test

The chromosomal patterns of bone marrow or blood cells have marked prognostic importance in MDS [51-54]. In ambiguous cases, the presence of an MDS-associated cytogenetic abnormality provides some security about the diagnosis [52]. Overall, about 40% of patients with \textit{de novo} MDS have an abnormal karyotype [55, 56]. In contrast, more than 85% of secondary MDS cases have a chromosomal abnormality-usually one with an extremely adverse prognosis, such as abnormalities of chromosome 11q23, or a complex karyotype [57- 59]. At present, even though point mutations can sometimes be detected in genes such as \textit{TP53}, \textit{NRAS}, \textit{FMS}, and \textit{RUNX1/AML1} in patients with MDS and their presence probably portends a poorer prognosis, there is usually no clinically compelling reason to test for them [53,60-64].

1.16. Classification of MDS

Characteristic features which are currently used to classify MDS are morphology of cells in blood and marrow, cytogenetics, and clinical features. The presence of abnormal types or numbers of cells in the bone marrow and/or blood is a hallmark of MDS and is a fundamental component of classification. The abnormalities include increased number of blasts, blasts with elongated rods called auer rods, ringed sideroblasts, red blood cell precursors that are haloed by an iron ring, malformations in nuclei and cytoplasm of red blood cells, white blood cells and platelet precursor cells, hyperplastic or hypercellular or hypocellular bone marrow. Combinations of morphological abnormalities and blood cell deficiencies have been recognized as distinct subtypes of MDS [65].

1.16.1. French American British (FAB) classification

The FAB classification system groups MDS into following subtypes based on percentage of blood and bone marrow blasts, ringed sideroblasts, monocytes, and presence or absence of auer rods as detailed below:
1.16.1.1. Refractory Anemia (RA)
Cytopenia of one peripheral blood lineage, normo or hypercellular marrow with dysplasia and <1% blasts in the peripheral blood and <5% bone marrow.

1.16.1.2. Refractory Anemia with Ringed Sideroblasts (RARS)
Cytopenia, dysplasia of more than one cell lineage, <1% blasts in the peripheral blood and <5% bone marrow and ringed sideroblasts account for >15% of nucleated cells in marrow.

1.16.1.3. Refractory Anemia with Excess Blasts (RAEB)
Cytopenia of two or more peripheral blood lineages, dysplasia of all 3 lineages, <5% peripheral blood blasts and 5-20% bone marrow blasts.

1.16.1.4. Refractory Anemia with Excess Blasts in transformation (RAEB-t)
Cytopenia of two or more peripheral blood lineages, dysplasia of all three lineages, >5% blasts in peripheral blood or 21-30% blasts in bone marrow or the presence of auer rods in the blasts.

1.16.1.5. Chronic Myelomonocytic Leukemia (CMML)
Monocytosis in peripheral blood (>1x10⁹/L), <5% blasts in peripheral blood and upto 20% bone marrow blasts.

1.16.1.6. Juvenile Myelomonocytic Leukemia (JMML)
The FAB classification has provided a common language for physicians in the investigation of myelodysplasia and also contributes prognostic information. The FAB system, although still used by clinicians, has several limitations regarding particular subgroups to new cytogenetic findings, treatment and prognosis [65]. Hence alternative classifications were proposed and continue to be revised.
1.16.2. World Health Organization (WHO) classification

Overcoming the controversies in the FAB classification, WHO in 2001 proposed a new classification system to improve the prognostic value of MDS patients (Table: 1.1). The major changes included are lowering the threshold of blast from 30% to 20%, in bone marrow/ peripheral blood, resulting in elimination of RAEB-t subtype in FAB category; division of the low grade categories of refractory anemia (RA) and refractory anemia with ringed sideroblasts (RARS) into 5 separate entities, depending on whether single lineage or multilineage dysplasia is present and an isolated interstitial deletion of chromosome 5q is present; subdividing RAEB into two categories depending on the number of blasts in the blood and marrow; removing CMML from the MDS category into a new group of diseases, the myelodysplastic/myeloproliferative diseases [66-69].

Although both the FAB and WHO classification systems for MDS offer general prognostic guidance, the International Prognostic Scoring System (IPSS) represents the first system to be accepted worldwide for routine management decisions. This model applies a score that is weighed according to the independent statistical power of each of three prognostic features: bone marrow blast percentage, cytogenetic pattern and the number of cytopenias. The cumulative score enables segregation of patients into four subgroups as low risk, intermediate risk 1, 2 and high risk, with varying expectations for survival and risk/interval to AML progression (Table:1.1) [66-71].
### Table: 1.1

Summary and comparison of MDS classification system

<table>
<thead>
<tr>
<th>FAB CLASSIFICATION</th>
<th>WHO CLASSIFICATION</th>
<th>IPSS CLASSIFICATION</th>
</tr>
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<tbody>
<tr>
<td>Refractory anemia (RA)</td>
<td>Refractory cytopenia with unilineage dysplasia (RCUD)</td>
<td>Marrow Blast Percentage</td>
</tr>
<tr>
<td>Refractory anemia with ringed sideroblasts (RARS)</td>
<td>Refractory anemia (RA)</td>
<td>Blast %</td>
</tr>
<tr>
<td>Refractory anemia with excess blasts (RAEB)</td>
<td>Refractory neutropenia (RN)</td>
<td>IPSS Score</td>
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<td>Refractory anemia with excess blasts in transformation (RAEB-t)</td>
<td>Refractory thrombocytopenia (RT)</td>
<td>&lt; 5</td>
</tr>
<tr>
<td></td>
<td>Refractory anemia with ringed sideroblasts (RARS)</td>
<td>5-10</td>
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<td></td>
<td>Refractory Cytopenia (MDS) with Multilineage Dysplasia (RCMD)</td>
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</tr>
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<td>Refractory Anemia with Excess Blasts type I (RAEB-I)</td>
<td>21-30</td>
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<tr>
<td></td>
<td>Refractory Anemia with Excess Blasts type II (RAEB-II)</td>
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<table>
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<tr>
<th>Cytogenetic Features</th>
<th>Karyotype</th>
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<tbody>
<tr>
<td></td>
<td>Good prognosis</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>(-Y, 5q-,-20q-)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Intermediate prognosis</td>
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</tr>
<tr>
<td></td>
<td>Poor prognosis</td>
<td>1.0</td>
</tr>
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(abnormalities of chromosome 7 and any complex karyotype (i.e. > 3 abnormalities)
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“Genetic study on refractory anemia group of myelodysplastic syndromes (MDS-RA)”
No: Ph.D/104-F.T/V/2006

<table>
<thead>
<tr>
<th>Chronic/Juvenile myelomonocytic leukemia (CMML/JMML)</th>
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<tr>
<td>MDS associated with isolated del(5q) Del(5q)</td>
</tr>
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<td>Childhood MDS, including refractory cytopenia of childhood (provisional) (RCC)</td>
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<tr>
<td>Myelodysplastic syndrome, unclassifiable (MDS-U)</td>
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<table>
<thead>
<tr>
<th>Cytopenias</th>
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<tbody>
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<td>Cytopenia</td>
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<tr>
<td>None or 1 type</td>
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<tr>
<td>2 or 3 type</td>
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**Overall IPSS score and Survival**

<table>
<thead>
<tr>
<th>Overall score</th>
<th>Median survival</th>
</tr>
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<tbody>
<tr>
<td>Low (0)</td>
<td>5.7yrs</td>
</tr>
<tr>
<td>Intermediate 1 (0.5 or 1)</td>
<td>3.5yrs</td>
</tr>
<tr>
<td>Intermediate 2 (1.5 or 2.0)</td>
<td>1.2yrs</td>
</tr>
<tr>
<td>High (&gt;2.0)</td>
<td>0.4yrs</td>
</tr>
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**IPSS Cytogenetic Classification:** Intermediate prognosis: +8, Single miscellaneous abnormality, double abnormalities;

**IPSS Types of Cytopenia:** Hemoglobin <10g/dL; Absolute neutrophil count <1500 per mm³; Platelet count < 100,000 per mm³.
1.17. Cytogenetics of MDS

In MDS, though large deletions with varied breakpoints and numerical chromosomal abnormalities predominate, and in most instances it is not yet clear which specific genes are critical and responsible for the phenotype. Clonal chromosomal aberrations are found in 30–50% of primary MDS and no specific cytogenetic abnormality was associated with a particular MDS subtype. These abnormalities were predominantly characterized by partial/total chromosomal losses or chromosomal gains. Among total chromosomal losses, monosomy of 5, 7 and Y were reported frequently, while partial chromosomal losses include mainly del(5q), del(7q), del(11q), del(17p) and del(20q) (Figure: 1.3) (Table: 1.2). Trisomy-8 represents the most common chromosomal gain. Interestingly, many of the recurrent abnormalities seen in MDS lead to the loss of genetic material in particular tumor suppressor genes, which normally function to control cell growth and/or cell death as regulating the cell cycle, repair of DNA damage and apoptosis [52,72-78].

Complex abnormalities are observed in 10–20% of patients with de novo MDS and in about 90% of patients with t-MDS [79,80]. Abnormalities involving chromosomes 5 and 7 were found in most of the cases with complex karyotypes. The detection of a cytogenetic abnormality may be useful to establish diagnosis in difficult cases of MDS, particularly when there is a change in clinical picture and unclear marrow findings. Additional chromosomal aberrations may evolve during the course of MDS or an abnormal clone may be developed in a patient with a previously normal karyotype. These changes often signal a change in the course of the disease usually to a more aggressive one and may herald incipient leukemia. Nonetheless, karyotypic evolution in MDS is associated with a transformation to acute leukemia in about 60% of cases and a reduced survival particularly for those patients, who evolve within a short period of time. Table: 1.2 summarizes the types of chromosomal abnormalities reported in MDS patients [81].
Although, many chromosomal abnormalities have been reported in the pathogenesis of MDS, the abnormalities that responsible for leukemic transformation are still unknown. However, chromosomal data of MDS patients help to guide therapeutic decisions.
### Table: 1.2
Common chromosomal abnormalities seen in myelodysplastic patients
(de novo MDS and t-MDS)

<table>
<thead>
<tr>
<th>Chromosome abnormalities</th>
<th>Genes implicated</th>
<th>Prognosis</th>
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<tr>
<td><strong>Chromosome 3</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>del(3p)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>inv(3)(q21;q26)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>t(1;3)(p36;q21)</td>
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<td></td>
</tr>
<tr>
<td>t(3;3)(q21;q26)</td>
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<td>t(3;5)(q25;q34)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>t(3;12)(q26;p13)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>t(3;21)(q26;q22)</td>
<td>EVI 1, MEL1</td>
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<tr>
<td><strong>Chromosome 5</strong></td>
<td></td>
<td></td>
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<tr>
<td>-5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>del(5)(q12-13;q31-33)</td>
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<td></td>
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<tr>
<td>del(5)(q12;q23)</td>
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<td></td>
</tr>
<tr>
<td>del(5)(q23;q32)</td>
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<td>Interleukin-6 (IL-6)</td>
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<tr>
<td>Interferon regulatory factor (IRF)</td>
<td>Granulocyte Monocyte Colony Stimulating Factor (GM-CSF)</td>
<td>Good</td>
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<td>Granulocyte Colony Stimulating Factor (M-CSF)</td>
<td>Early Growth Response (EGR-1)</td>
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<tr>
<td><strong>Chromosome 7</strong></td>
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<tr>
<td>-7</td>
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<tr>
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<td>del(7)(q31-q36)</td>
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<tr>
<td>T cell receptor-β (TCR-β), Erythropoietin (EPO), Asparagine synthase gene (ASNS), PIK3KG</td>
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<td><strong>Chromosome 8</strong></td>
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<td>+8</td>
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<td>11p15</td>
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<tr>
<td>inv(11)(p15q22)</td>
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<tr>
<td>t(2;11)(q31;p15)</td>
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<td></td>
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<tr>
<td>t(11;17)(p15;q21)</td>
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<tr>
<td>t(11;20)(p15;q11)</td>
<td>NUP98, HOXA9, HOXD13, HOXB, HOXC, TOP1, DDX10</td>
<td>Poor</td>
</tr>
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</table>
Despite the importance of karyotyping, sometimes due to technical difficulties like low mitotic index and the poor quality of the metaphases, clonal chromosomal abnormalities occurring in minority of cells, conventional cytogenetics cannot be informative in MDS. A number of studies have postulated the superiority of Fluorescent In Situ Hybridization (FISH) as the alternative as, this technique permit examination of a large number of cells and specific chromosomal aberrations in interphase as well as in metaphases. Ketterling et al [82], applied M-FISH or interphase FISH using multiple probes to detect -5/5q-, -7/7q-, 17p-, 12p-, 13q-, 20q-, +8, +21 and abnormalities of chromosome 11 on MDS patients who had a normal karyotype by conventional cytogenetics and suggested that conventional cytogenetic analysis identifies the overwhelming majority of cytogenetic...
aberrations in patients with MDS. Mohr et al [83], studied 39 patients with MDS or AML by conventional cytogenetics and spectral karyotyping technique (SKY). In the patients with a normal karyotype no abnormal metaphases were detected by SKY. However, in patients with single aberrations or complex karyotypes multicolor painting techniques could have the advantage to detect hidden abnormalities. Wilkens et al [84], compared comparative genomic hybridization (CGH) results with conventional cytogenetics and FISH using probes to detect 5q31, 20q13, chromosomes 7, 8, 18, 21, X and Y in 45 MDS patients. In two cases, the results of conventional cytogenetics and CGH were divergent while one of them conventional cytogenetics did not show abnormalities by banding while the CGH defined these abnormalities as 5q- and -7. In the second case a t(3;7) detected by conventional cytogenetics was not found by CGH. In all patients in whom conventional cytogenetics and/or CGH reveal chromosome imbalances the FISH confirmed these aberrations. However, due to high cost of molecular cytogenetics testing and the limitations, conventional cytogenetics cannot be excluded nor replaced as part of diagnostic/prognostic tool in myelodysplasia patients. Thus a combination of conventional cytogenetics and FISH technique could lead to a more accurate diagnosis. Moreover, a detailed molecular analysis to understand the disease etiology, pathogenesis and progression would enable a better approach to prognostication of myelodysplastic patients.

1.18. Molecular pathogenesis:

In spite of a multiplicity of endeavors to elucidate the molecular mechanisms of MDS, little is known about the pathogenesis of the first trigger or the early stage of MDS. Based on the accumulated knowledge on the genes involved in chromosomal translocations and somatic mutations, several molecules and signal transduction pathways were proposed as an initiating step in oncogenesis.

In relation to oncogenesis, one of the notable signal transduction pathways is the RAS, since RAS oncogene product is one of a key molecule in the
development of wide variety of tumors including MDS as well as leukemias [85]. Normal cell growth through the cell cycle, is regulated by sequential formation, activation and subsequent inactivation of a series of cyclin-dependent kinase (CDK) complexes. The mechanisms underlying the expression of cyclins and activation of the different cyclin-CDK complexes required for progression through successive cell cycle transitions are now well understood. In addition to positive regulation by activation of cyclin-CDK complexes, negative regulation is also in operation at several checkpoints within cell cycle. It is now well known that tumors can be caused by loss of the normal breaks, the tumor-suppressor genes. The $^{53}$P stimulates production of $^{21}$P which blocks cyclin–CDK complexes and therefore causes G1 arrest [86]. Similarly, $^{15}$INK4B, $^{16}$INK4A and RB form complexes with cyclin or CDK and contribute to cell growth suppression.

Among the cell cycle regulators, $^{15}$INK4B is most frequently and $^{53}$P less frequently involved in pathogenesis of MDS, at least in the later stage of the disease. In terms of pathogenesis, initial development, the most highlighted molecules are nuclear proteins, especially transcription factors that tightly regulate cellular development and cell lineage-specific gene expressions. Among the genes encoding transcriptional regulators, of which alterations are detected in MDS or MDS/AML, are $AML1$, $C/EBP\alpha$ TEL (ETV6), $MLL$ and $EVI-1$. $AML1$, $C/EBP\alpha$ and TEL (ETV6); they were demonstrated to be essential in the hematopoietic cell development or differentiation by the gene targeting method in mice. Few of the genes that are implicated in the pathogenesis of MDS were studied in detail and has been reported but with low incidence and inconsistency [Table 1.3] [87-89].
Table: 1.3
Genes implicated in myelodysplasia

<table>
<thead>
<tr>
<th>Gene implicated</th>
<th>Molecular defect</th>
<th>Incidence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CDKN2B</td>
<td>Promoter methylation</td>
<td>30-50</td>
</tr>
<tr>
<td>EVI1</td>
<td>Ectopic expression</td>
<td>30-40</td>
</tr>
<tr>
<td>NPM1</td>
<td>Translocation, mutation</td>
<td>30</td>
</tr>
<tr>
<td>FLT3</td>
<td>Tandem duplications</td>
<td>25-30</td>
</tr>
<tr>
<td>IRF1</td>
<td>Exon skipping</td>
<td>20-30</td>
</tr>
<tr>
<td>BAALC</td>
<td>Over expression</td>
<td>25</td>
</tr>
<tr>
<td>ERG</td>
<td>Over expression</td>
<td>16</td>
</tr>
<tr>
<td>KRAS</td>
<td>Point mutation</td>
<td>10-15</td>
</tr>
<tr>
<td>NRAS</td>
<td>Point mutation</td>
<td>10-15</td>
</tr>
<tr>
<td>WT1</td>
<td>Mutation, over expression</td>
<td>10-15</td>
</tr>
<tr>
<td>MLL</td>
<td>Tandem duplication</td>
<td>11</td>
</tr>
<tr>
<td>CEPBA</td>
<td>Mutation</td>
<td>10</td>
</tr>
<tr>
<td>KIT</td>
<td>Point mutation</td>
<td>10</td>
</tr>
<tr>
<td>RUNX1</td>
<td>Mutation</td>
<td>6-10</td>
</tr>
<tr>
<td>TP53</td>
<td>Mutation, deletion</td>
<td>5-10</td>
</tr>
<tr>
<td>MLL</td>
<td>Amplification</td>
<td>5</td>
</tr>
<tr>
<td>NF1</td>
<td>Mutation</td>
<td>Rare</td>
</tr>
<tr>
<td>CSF1R</td>
<td>Mutation</td>
<td>Rare</td>
</tr>
<tr>
<td>CDKN2A</td>
<td>Mutation, deletion</td>
<td>Rare</td>
</tr>
<tr>
<td>RB1</td>
<td>Mutation, deletion, promoter methylation</td>
<td>Rare</td>
</tr>
</tbody>
</table>
1.18.1. **Ras**

*RAS* is an important signaling component for cell proliferation and is activated by receptor tyrosine kinases (*RTKs*) stimulated with extracellular ligands [90]. *RAS* has the *GTP*-binding activity and the *GTP*-hydrolyzing property. The inactive form of *RAS/GDP* is converted to an active form of *RAS/GTP* by guanine nucleotide-exchange protein (*GEP*) upon activation of RTKs with extracellular ligand stimuli. *RAS/GTP* interacts with target proteins such as *RAF*, thereby activates the downstream signaling molecules, *MAPKs*, and is converted to an inactive form *RAS/GDP* by hydrolysis of bound GTP in the presence of GTPase-activating protein. Mutated *RAS* proteins do not show GTPase activity, thus accumulate *RAS/GTP* and thereby constitutively activate the downstream signaling. *RAS* genes are known to be activated by point mutations frequently at codons 12, 13 or 61 [87]. Among *RAS* genes, mutations of the *N-ras* are most frequent and detected in 20–30% of human leukemias and 10–15% of MDS cases. These observations suggest that activation of *N-ras* should be related to leukemic transformation at least in a fraction of MDS patients.

1.18.2. **FLT3**

*FLT3* gene encodes receptor-type tyrosine kinase activity that is involved in proliferation and differentiation of hematopoietic precursor cells. An internal tandem duplication of the human *FLT3* was found as a somatic mutation in 15–20% of AML and 5% of MDS [88]. This abnormality seems to be a late event during the pathogenesis and patients with *FLT3* mutations tend to have a poor prognosis; it was suggested that *FLT3* tandem duplication might be associated with leukemic transformation from antecedent MDS [89].

1.18.3. **P$^{53}$**

The *P$^{53}$* is a hallmark of tumor-suppressor genes and its alterations are involved in various types of human malignancies. Inactivation of the *P$^{53}$* in
both alleles by mutations or deletions has been shown to predispose the cells to neoplastic transformation. Inactivation of the $P^{53}$ was detected in 5–10% in clinically advanced stages and in karyotypically unstable cases with MDS, indicating that $P^{53}$ mutations may play a role in leukemic progression of MDS [91].

1.18.4. *AML1*

The *AML1 (Runx1)* gene encoding the heterodimeric transcription factor, which binds to DNA through the Runt domain, is frequently involved in chromosomal translocations associated with human leukemias. Heterozygous mis-sense mutation of the *AML1* is noted to be causative for familial platelet disorder (FPD) with predisposition to AML; haploinsufficiency of *AML1* causes an autosomal dominant congenital platelet defect and predisposes to the acquisition of additional mutations that cause leukaemia [92, 93], reported that mis-sense mutations of the *AML1*, at Runt domain, in ~5% of AML subjects. It is notable that *AML1* mutations are preferentially detected in M₀ phenotype of AML, with high frequencies of 22% [94]. Although less frequently, the *AML1* is also a target of mutations in MDS and at least some of them show not only a loss-of function phenotype of AML1 but also a dominant negative effect on normal *AML1* function [95].

1.18.5. *C/EBPα* gene

The transcription factor *C/EBPα* (for CCAAT/enhancer binding protein-alpha) is crucial for the differentiation of granulocytes. Conditional expression of *C/EBPα* triggers neutrophilic differentiation and no mature granulocytes are observed in *C/EBPα*-mutant mice. Heterozygous mutations in *C/EBPα* are found in 7–8% of AML and rarely in MDS [96,97].
1.19. Apoptosis in Myelodysplasia

An apparent paradox in MDS is that patients with these disorders have peripheral cytopenias despite frequently having normo or hypercellular bone marrows. One possible explanation for this contradictory finding may be that even though there are large numbers of cells in the bone marrow, they are not exiting from that compartment because they are undergoing premature programmed cell death (PCD). It has been demonstrated that MDS patients had significantly higher apoptosis than control [98, 99], supports the fact that higher rate of apoptosis resulted in a functionally aplastic condition in patients despite hypercellular marrows; further cells undergoing apoptosis were differentiating cells rather than the hematopoietic cells. Among MDS, RARS sub-groups showed the most prominent ineffective erythropoiesis [100,101]. The role of apoptosis in MDS was also supported by in-vitro studies using cell lines; the MDS derived cell line P39 shows highest sensitivity to apoptotic stimuli among human leukemia cell lines [102].

1.19.1. Regulation of apoptosis

As discussed in earlier that the apoptosis, a proposed mechanism for the observed cytopenia in MDS cases could be mediated by the orchestra of many signaling molecules which are either pro-inflammatory or anti-inflammatory in nature, as reported for other tumourogenesis. They are discussed in detail in relevance to MDS in the following paragraphs:

1.19.1.1. Tumour Necrosis Factor-alpha (TNF-α)

TNF-α is secreted by several types of cells [103-106]. In general, a negative feedback loop prevents the over-expression of TNF-α in healthy subjects; whereas, in MDS patients, this negative feedback loop was disrupted, resulting in increased TNF-α over expression. The abnormally high levels of TNF-α promote apoptosis in haematopoietic cells during the early stages of MDS, contributing to the classic findings of ineffective haematopoiesis and
peripheral blood cytopenia [107-111] has reported, CD14\(^+\) monocytes, which are part of the MDS clone, as an important source of \(TNF-\alpha\) in MDS patient. However, over a period of time, the malignant clones acquire resistance to the pro-apoptotic effects of \(TNF-\alpha\), results to uncontrolled proliferation and transformation into the more aggressive stages of MDS. Recent studies have found that \(TNF-\alpha\) inhibitors improve pancytopenia in some MDS patients, provided indirect evidence on the involvement of that \(TNF-\alpha\) is involved in the pathogenesis of MDS [112-115].

IL32 has recently been identified as a membrane-associated pro-inflammatory and pro-apoptotic cytokine [116, 117], and studies have linked its expression to autoimmune diseases [118,119]. *In vitro* studies have shown that IL32 induces the expression of several pro-inflammatory genes (e.g. \(TNF, IL6\) and \(IL8\)) in haematopoietic cells [116,117]. Gene profiling analyses indicate that IL32 mRNA is, indeed, up-regulated in marrow cells in subgroups of patients with MDS. These findings, suggest that IL32 and TNF may be participating in an auto-amplification loop [Figure: 1.4].

**Figure: 1.4**

Pathways of \(TNF-\alpha\) mediated apoptosis

Source: [120, 121]

"Genetic study on refractory anemia group of myelodysplastic syndromes (MDS-RA)"
1.19.1.2. Transforming Growth Factor-beta (TGF-β)

TGF-β is the prototype of extracellular ligands that form the TGF-β superfamily of morphogenetic factors, additionally including bone morphogenetic proteins, growth differentiation factors, mullerian inhibiting substance, activins and others [122,123]. Originally, TGF-β was discovered as a secreted polypeptide factor from chemically or virally transformed fibroblasts that could elicit transformation of normal fibroblasts in classical in vitro assays [124,125]. Later, it was shown that TGF-β also acts as an inhibitor of cell proliferation, cell growth control; however, those functions are cell type-dependent [126,127]. In addition, this growth factor was rapidly established to affect a large variety of cellular processes during embryonic development and adult tissue homeostasis [128]. TGF-β is known to act as a tumor suppressor in early stages of tumorigenesis, by inducing the production of mitogenic factors such as hepatocyte growth factor/scatter factor or platelet derived growth factor in the tumor stroma [129] and it can also promote advanced tumor cell invasiveness and metastasis. The TGF-β pathway is targeted by loss-of-function mutations in various human cancers, thus relieving tumor cell growth from a major cytostatic agent and an inducer of apoptosis of diverse cell types [130].

Inherent to the tumor suppressor action of TGF-β is its ability to induce apoptosis in a cell type-specific manner, and mutations in components of the signaling pathway are thought to contribute to resistance to pro-apoptotic responses in Smad dependent and independent manner as shown in Figure: 1.5. Also, upon long-term exposure to TGF-β, a great variety of cell types inactivate the NF-κB pathway, via transcriptional induction of the inhibitor of NF-κB (IκBα), thus favoring a pro-apoptotic fate [131-133]. In addition, TGF-β, in a Smad3-dependent manner induces expression and activates the Fas receptor, without affecting its cognate Fas ligand, leading to caspase-8 activation and apoptosis of gastric carcinoma cells [134].
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TβRII directly associates with the Fas receptor adaptor protein Daxx, which mediates JNK activation in response to TGF-β and during apoptosis of lymphocytes or hepatocytes [135]. TGF-β-activated JNK cross talks with Smad3 and Smad4 in regulation of gene expression, as it phosphorylates Jun family members that interact with the Smads and bind to regulatory sequences of genes involved in eliciting the apoptotic response [136,137,138]. In short, the apoptotic response of normal or tumor cells to TGF-β is complex, incorporating both pro-survival and pro-apoptotic pathways and a series of cytoplasmic and nuclear effectors. In all cases, the net decision of whether TGF-β will elicit apoptosis or might favor survival depends on alternative signaling inputs that the cell receives (Figure: 1.5).

1.19.1.3. Fas

Fas is a type I membrane protein belonging to the TNFR superfamily, which includes the P55 (TNFR1), P75 (TNFR2) TNFRs, CD40, 4-1BB, and the family of TNF-related apoptosis-inducing ligand receptors [139,140]. Constitutive Fas ligand (FasL) expression helps to maintain immune privilege and immune deviation [141,142], development of the immune response, homeostasis of the immune system, and maintenance of self-tolerance [143, 144]. Additionally, Fas-mediated apoptosis is involved in T cell cytotoxicity, tumorigenesis, and liver disease [145-147]. Cross-linking of Fas by FasL or specific antibodies initiates the cell death cascade, with the binding of Fas-associated death domain protein (FADD) to the cytoplasmic domain [148,149]. This facilitates the binding and activation of caspase-8, and its downstream caspases, degradation of DNA, and ultimately cell death [134]. Interestingly, molecules like IL-2 [150] potentiate Fas-mediated death through the modulation of apoptosis regulatory protein the FADD-like IL-1β-converting enzyme inhibitory protein (FLIP) [Figure 1.6] [151]. The resistance of naive T cells to Fas-mediated death has been attributed to high intracellular levels of FLIP, which decrease following cellular activation and IL-2 production, thereby making the T cells susceptible to Fas-mediated death [151,152].
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No: Ph.D/104-F.T/V/2006

Figure: 1.5

TGFβ signaling pathway of apoptosis

Source: [123, 137]
Several investigators have reported an increased marrow cell apoptosis in MDS and have implicated the potential role of the Fas/Fas- L system [98, 153-157]. Increased Fas expression was detected on marrow CD34 cells from MDS patients [120,154-157]. In parallel, lack of correlation between Fas expression and apoptosis suggests that multiple mediators of cell death must be are operational [155]. Gupta et al [158], have examined Fas ligand expression in marrows of MDS patients which was higher in MDS (17%) than from normal controls (6%); in contrast to normal individuals, where Fas ligand was detected mostly in lymphocytes, it was expressed in erythroblasts, myeloblasts, megakaryocytes, maturing myeloid cells and dysplastic cells in MDS patients. In another study, Fas ligand expression in MDS patients was localized to marrow macrophages [159].

Further investigations revealed more Fas-L positive marrow cells in RA/RARS (9%) than in RAEB/RAEBt (20%) [158]. However, this appears to be inconsistent with the finding that the extent of marrow cell apoptosis inversely correlates with clinical stage of MDS. Recent studies have shown that Fas-associated phosphatase- 1 (fap-1), a negative regulator of Fas, which expression was reduced in MDS marrow cells compared to marrow cells from either normal or AML subjects than has progressed from MDS [160]. Several investigators have suggested that the clinical sequel of apoptosis is cytopenia. Since MDS is usually detected by cytopenia and apoptotic activity is most pronounced in the early phases of MDS, it is possible that apoptosis precedes the clinical recognition of MDS.

Studies have shown that noncytotoxic levels of TNF may regulate Fas-mediated apoptosis through the modulation of two important intracellular regulators of apoptosis. The sensitizing signal that TNF transmits to the cell down-regulates an antiapoptotic protein (FLIP), while up-regulating a proapoptotic one (Bax), enabling a signal through Fas to activate the cellular apoptotic pathway. TNF affects several components of the cell death machinery. TNF-TNFR2 interaction alters the expression of the intracellular regulators of apoptosis, Bax and FLIP. Bax is a pro-apoptotic member of the
Bcl-2 family of proteins that regulates cell death through the formation of homodimers and heterodimers with Bcl-2. It has been suggested that the ratio of Bcl-2:Bax determines cell survival or death following an apoptotic stimulus [161-164].

**Figure: 1.6**

*Fas mediated apoptosis [134]*

1.19.1.4. Interferon (IFN-γ)

The IFNs were originally discovered as agents that interfere with viral replication [165]. Initially, they were classified by the secreting cell type but are now classified into type I and type II according to receptor specificity and sequence homology. One of the most easily observed effects of IFN-γ is cell growth inhibition. Types I and II IFN are able to protect against pathogen-induced apoptosis and suppress colony stimulating factor type 1 (CSF-1)-dependent growth of bone marrow-derived macrophages (BMM) [169]. The IFNs most predominantly arrest the cells at the G1/S checkpoint, [166,167,168]. IFNs inhibit cell proliferation primarily by increasing protein
levels of INK4 and Cip/Kip CKIs [169-174]. IFN-γ transcriptionally induces P21 and P27 CKIs [175-177], which in turn inhibit the activity of cyclin E:CDK2 and cyclin D:CDK4 complexes, respectively, and arresting the cell cycle at the G1/S boundary. Expression level of c-myc, another molecule involved in the regulation of G1/S phase transition, was influenced by IFN-γ [178,179]. IFN-γ influences c-myc expression through multiple pathways, including suppression of Rb phosphorylation and resulting in decreased E2F activity as well as Rb-independent pathways [180]. In its active form, c-myc is associated with max, and the myc:max heterodimer activates transcription of genes required for cell cycle progression [181]. The level of active myc is negatively regulated by mad1, which sequesters the max coactivator away from myc and suppresses transcription of myc-inducible genes [182]. In this way, the mad1:c-myc ratio determines whether a cell do proliferate or arrest in G1 [182,183]. In addition to decreasing the abundance of myc, IFN-γ increases mad1 levels, thereby further antagonizing myc activity and inhibiting CSF-1-dependent proliferation in BMM [184]. It was also been suggested that IFN-γ or IL-1β is an apoptogenic cytokines that could contribute to ineffective hematopoiesis in MDS. Contradictory findings were observed on the IFN-γ gene over-expression [185,186]; however, a clear increased production of IL-1β was shown in blood and marrow cells of MDS patients [187,188]. Patients with RA tended to have the highest IL-1β production [187,189] and the IL-1β levels have been correlated with the extent of apoptosis, but not proliferation [187]. The involvement of IL-1β was further supported indirectly that, deficient production of the IL-1β receptor antagonist by MDS stromal cells may give rise to unopposed apoptotic activity from IL-1β [190].

1.20. Polymorphism of cytokines and their receptors

Most genetic variation comprises single base changes in the DNA sequence, known as single nucleotide polymorphisms (SNPs), occurring approximately once every 1000 nucleotides [191,192] both coding and non-coding regions. If it occurs in coding DNA, the implications may be readily apparent as a change in the amino acid make-up of the translated protein [193]. In contrast, assaying
the functional effect of polymorphisms occurring in non-coding DNA mainly at putative regulatory may affect the process of gene expression. The cytokine gene families are highly polymorphic within their coding sequences and in sequences regulating their expression, some of which are known to modify cytokine activity. Studies have sought to identify polymorphism genes codes for cytokine receptors. The obtained results showed that cytokine and cytokine receptor genes are highly conserved at their exons and majority of polymorphism are reported in non-translated regions of the gene including promoters and introns. Polymorphisms have been identified in the upstream regulatory regions of \( \text{TNF-\alpha} \) and within the leader peptide sequence of \( \text{TGFRII} \) [194-196]. Under certain experimental situations, the -308A and -238A polymorphisms in \( \text{TNF-\alpha} \) and the polymorphisms that encode a leucine at amino acid 10 and an arginine at amino acid 25 in \( \text{TGF-\alpha} \) are associated with increased expression [197,198]. A recent study described 28 polymorphisms in the \( \text{IL-10} \) gene, 14 with a frequency >5 % [199]. Even cytokines that were originally thought to be non-polymorphic, such as \( \text{IL-2, IL-8} \) and \( \text{IL-12} \), are now being shown to possess SNPs often within their 5’ promoter regions [200-202]. Lazarus et al [199], described 28 polymorphisms in the \( \text{IL-10} \) gene, of which none involved an amino acid substitution.

### 1.20.1. The effects of polymorphism on cytokine production

The vast majority of polymorphisms in cytokine genes are either located in the non-translated regions of the gene or involve silent mutations of exons. Conservative mutation may still influence levels of protein expression in a number of ways. Polymorphism within 5’ and 3’ regulatory sequences may affect transcription by altering the structure of transcription factor binding sites. Intronic polymorphism may affect mRNA splicing or the structure of enhancers or silencers. Finally, polymorphism may alter the structure of binding sites for architectural transcription factors that are known to modulate promoter activity [203]. These effects of conservative mutation often have less impact in terms of cytokine production levels than those that alter the structure of the protein. Studies looking at the effects of the same polymorphic loci in
the same genes often give conflicting results [196,204,205]. If increases or decreases in cytokine activity promote the development of MDS, it is likely that polymorphisms in such cytokine genes that modify their expression and/or activity will be associated with an increased risk of disease and therefore overrepresented in an MDS population.

Previous studies have associated a polymorphism in TGF-α with more severe anemia in patients with refractory anemia, but two other studies have not identified an association between polymorphisms in TNF-α and MDS [206,207]. In two MDS patients, serum levels of IL-6 was found high, but polymorphism study revealed no significant association between expression and polymorphism of IL6 in controls and subjects. A number of reasons may explain the contradictions in the cytokine expression studies. Although, numerous studies have been carried out on MDS cases or on cell lines from MDS marrow, the drawback was the MDS disease heterogeneity. However, studies so far have tried to look at the insight of the disease pathogenesis and progressed considerable to unveil the possible mechanisms that could have been the cause of disease etiology.

1.21. Multistep pathogenesis

Three decades of investigations into the pathophysiology of the MDS have confirmed the heterogenicity of MDS and highlighted the complexity in disease biology [208]. A specific multistep sequence for the development of adult-onset idiopathic MDS based on cell culture, cytokine, molecular and clinical research was proposed. The progenitor cells damaged by toxin exposure or spontaneous mutation evoke an immunologic response that further compromises progenitor cell growth and maturation. Clinical observations support the notion of immune suppression of progenitor cell growth in MDS. Non-clonal lymphopoiesis provides indirect evidence for a lack of stem cell involvement in MDS. Using precursors sorted by flow cytometry and subsequent FISH to define clonal hemopoiesis, primitive progenitors (CD34+, Thy1+) lacked the cytogenetic marker whereas more committed progenitors
(CD34+, CD33+) display a clonal chromosome abnormality [209]. The growth and differentiation of the progeny of clonal progenitors may further compromised by an accelerated rate of apoptotic cell death.

1.22. **Hypothesis and objectives of the study**

In late MDS, more than extensive apoptosis, clonal evolution and expansion explains involvement of molecular changes enabling increased cell proliferation resulting in leukemic transformation. Although, in early MDS it is still not clear about the initiating event causing disease pathogenesis, the apoptosis is considered as one of the major criteria apart from cell maturation/differentiation impairment and arrest of cell proliferation. As apoptosis is regulated by the variety of signaling pathways and signaling molecules, we strongly believe and hypothesize that altered cytokines expression could alter signaling pathway towards increased apoptosis in early MDS [Figure: 1.7].
The objectives of the current study are as follows:

a) Estimation of rate of apoptosis in patients diagnosed as myelodysplastic syndrome with refractory anemia (MDS-RA).

b) To karyotype the MDS-RA patients included in this study and look for any abnormality that could implicate the disease pathogenesis.

c) Estimation of pro-inflamatory cytokine levels such as GAPDH, TNFa, TNFRI, TNFRII, TGF-β1, TGF-β2, TGF-β3, Fas-L, Fas-R, IFN-γ in bone marrow samples of MDS-RA.

d) To study the expression of transcription factors NFκB1, NFκB2, NFκB and correlate the levels with levels of control samples.

e) To study the influence of polymorphisms over cytokine expression levels.

Figure: 1.7

Hypothesis on pathogenesis of myelodysplasia
1.23. References


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[99] Yoshida Y. Hypothesis: apoptosis may be the mechanism responsible for the premature intramedullary cell death in the MDS. Leukemia. 1993; 7(1):144-6.


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[148] Boldin MP, Varfolomeev EE, Pancer Z, Mett IL, Camonis JH, Wallach D. A novel protein that interacts with the death domain of Fas/APO1


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