INTRODUCTION

Myelodysplastic syndrome (MDS) is a clonal disorder at the multipotent, haematopoetic stem leve ***(Knuutila, 1997)***. The bone marrow is usually normocellular or hypocellular, indicating a high degree of ineffective hematopoiesis ***(Ikuo-Miura et al., 2000)***.

Therapy related acute leukemia and MDS occur in patients who have received chemotherapy for neoplastic or non-neoplastic disorder. The major types of drugs associated with therapy related acute myeloid leukemia (AML) are alkylating agents and type II topisomerase inhibitor drugs ***(Coiffier, 1983)***.

Monosomy of chromosomes 5 and 7 or delation of their long arms [5/del (5q); -7/del (7q)] are frequently observed chromosomal abnormalities with the novo MDS, in which each is observed in about 30% of the cases ***(Vallespi et al., 1998)***.

Therapy-related MDS (t-MDS) and therapy-related AML (t-AML) are associated with a higher frequency of karyotypic abnormalities after including -5/del (5q) and -7/del (7q). Nearly 71% of patients with t-MDS/t-AML have abnormalities of chromosomes 5 and/or 7 ***(Le Beau et al., 1997)***.

Loss of the long arm, 5q- , or complete loss of the entire chromosome 5 (-5), have a dual clinical significance. On the one hand, 5q- as a sole abnormality in a patient with referactory anaemia is the hallmark of the 5q- syndrome. This syndrome has a relatively good prognosis, with transfusion dependency being the primary clinical manifestation; transformation into acute myeloid leukemia (AML) in infrequent ***(Greenberg, 1999)***.

On the other hand, the co-existence of a chromosome 5 delation with additional karyotypic abnormalities, or after prior chemotherapy, have a poor clinical prognosis and rapid transformation to AML ***(Greenberg et al., 1999)***. The AML that arises after this transformation generally responds poorly to chemotherapy, with rapid progression and short survival ***(Pedersen-Bjergaard et al., 1990)***.

The presence of a chromosome 5 delation is thus an important prognostic factor in the management of MDS ***(Heaney and Golde, 1999)***.

Fluorescence in situ hybridization (FISH) has become the major tool in cytogenitic and pathology laboratory because it is a rapid technique, high efficiency of hybridization and detection, high specifity and sensitivity, large number of cells can analysed in a short time and cytogenitic data can be obtained from non-dividing or terminal differentiated cells, or from poor samples that contain too few cells for valuable cytogenitic studies ***(Saitoh et al., 1998)***.

AIM OF THE WORK

The aim of this study is to show significance of fluorescence in situ hybridization (FISH) in detection of chromosomal abnormalities of chromosome 5 and 7 in myelodysplastic syndrome (MDS) and secondary leukemias. As well as to investigate the correlation of theis chromosomal abnormalities with clinical outcome.

MYELODYSPLASTIC SYNDROME

The myelodysplastic syndromes (MDSs) are group of bone marrow disorders characterizes by dysplastic changes in one or more myeloid cell lines with or without concurrent increase in myeloblasts in the bone marrow and peripheral blood, in those instances in which the meyloblasts are increased, their number is less than the 20% ***(Brunning, 2001)***.

The MDSs occur as primary disease and as secondary or therapy-related disorders. The therapy-related MDSs occur in patients who have been exposed to chemotherapeutic agents or radiotherapy ***(Aul et al., 2001).***

Pathogenesis:

MDS is a clonal disorder of hematopoiesis with site of transformation at a pleuripotent stem cell or early myeloid progenitor cells ***(Kouides and Bennett, 2000)***.

Molecular genetic studies of MDS patients showed identifiable gene mutation in about 60% of patients. Mutated RAS is the most common, lower frequencies of FMS and p53 mutations are present. The resultant genetic alterations may be manifested by increased apoptosis ***(Plata et al., 1999).***

The consequence of bone marrow stem cell dysfunction is the failure to produce peripheral blood cells. This may be secondary to either a lack of differentiation of the stem cell and/or an excess amount of apoptosis in the bone marrow ***(Parker and Mufti, 1999)*.**

MDSs are characterized by the paradox of peripheral blood cytopenias in the presence of a hypercellular bone marrow. The hypothesis that MDS is characterized by excessive apoptosis may provide an intriguing explanation for this apparent paradox. Part of the molecular pathology of apoptosis includes DNA fragmentation; the amount of 5` DNA ends in a sample can be quantitated as a surrogate marker of the rate of apoptosis. Most bone marrow samples from patients with MDS also exhibit increased rates of cell growth. This data lead to a hypercellular bone marrow, but increased rates of apoptosis render the hypercellular bone marrow ineffective with resultant peripheral blood cytopenias.

Cytokine dysregulation may also play an important role in the development of MDS. Some patients with MDS have low levels of haematopoietic cytokines. Approximately 20% of patients with MDS have inappropriately low levels of erythropoietin for the degree of anemia. In addition, MDS patients have been found to have high levels of cytokines that could promote apoptosis, such as interleukin-1B (IL-1B),

tumour necrosis factor-(TNF-) and transforming growth factor-B (TGF-B). Intriguingly, all of these cytokines probably share a common lipid-based intracellular signaling pathway which involves diacylglycerol metabolism. Regardless of the imbalance between cell proliferation and apoptosis in MDS, a patient with this condition will probably succumb to the disease. In approximately 30-40% of patients with MDS, the bone marrow evolves to AML ***(Parker and Mutfi, 1999)***.

Classification:

The de novo MDSs may be classified into three category depend on the French-Amircan-Brithish morphology group (FAB), World Health Organization (WHO), and the International Prognostic Scoring System (IPSS) which there are a disparate methods to evaluate the potential clinical outcomes for patients with MDS (Table 1) ***(Mufti et al., 2004)***.

Table (1). MDS classification for prognosis and survival as determined by FAB, WHO, and IPSS.

Table 1. MDS classification for prognosis and survival as determined by FAB, WHO, and IPSS.

Patient characteristics		Classification			
				IPSS	
Blood findings	Bone marrow	FAB	WHO	Score	Karyotype cytopenias cytogenetics
Anaemia, no or rare blasts, no Auer rods	Erythroid dysplasia only; <5% blasts; <15% ringed sideroblasts; no Auer rods	Low grade MDSRA	RA	0	*Good; 0/1; del (5q), del (20q), -Y
Anaemia only, no blasts/Auer rods	As above, but ≥15% ringed sideroblasts	RARS	RARS		
Cytopenias (2–3), nor rare blasts, no Auer rods, <1 000 monocytes/μl	Dysplasia in ≥10% of cells in ≥2 lines or similar to RA	RA	RCMD		
Cytopenias (2–3), nor rare blasts, no Auer rods, <1 000 monocytes/μl	As in RMCD except ≥15% ringed sideroblasts	RA	RCMD and ringed sideroblasts		INT-1 or INT-2 with CD34-positive staining or †ALIP-†poor
Cytopenias, <5% blasts, no Auer rods, <1 000 monocytes/μl	Uni or multilineage dysplasia, 5–9% blasts, no Auer rods	RAEB	RA with excess blasts-1	0.5	Intermediate 2/3 other
Cytopenias, <5% blasts	Uni or multilineage dysplasia, <10% blasts			1	*Poor; -7, del (7q) ≥3 abnormalities
Cytopenias, 5–19% blasts, Auer rods, ±1 000 monocytes/μl	Uni or multilineage dysplasia, 10–19% blasts, ±Auer rods	RAEB	RA with excess blasts-2	1.5	Poor; 11–20% blasts
Cytopenias (2–3), nor rare blasts, no Auer rods, <1 000 monocytes/μl	Unilineage dysplasia, <5% blasts, no Auer rods	–	Unclassified MDS		
Anaemia, normal or increased platelet count, <5% blasts	Normal to increased dysplastic megakaryocytes, <5% blasts, no Auer rods	–	MDS associated with isolated del (5q)		
Cytopenias (Hg < 10 g/dl Neutrophil count <1 500/μl, platelet count <100 000/μl)				2.0	Poor; 21–30% blasts

KEY: FAB, French–American–British[9]; WHO, World Health Organization[16,17]; IPSS, International Prognostic Scoring System[19,22,23,96]; RA, refractory anaemia; RAEB, refractory anaemia with excess blasts; RARS, refractory anaemia with ringed sideroblasts; RCMD, refractory cytopenia with multilineage dysplasia. IPSS low, 0; IPSS intermediate-1 = 0.5–1; IPSS intermediate-2 = 1.5–2.0; IPSS high >2.5 high. Karyotypes: *Good = normal, -Y, del(5q), del(20q); *Poor = complex (≥3 abnormalities) or chromosome 7 abnormalities; intermediate = other karyotypic abnormalities[22,23]; †Poor-50% incidence of developing leukaemia.[28] ALIP, abnormal localization of immature precursors.

(Mufti et al., 2004)

1-Refractory anemia:

Anemia refractory to hematenic therapy is the major finding. The red cells usually are normochromic and macrocytic but may be normocytic. The degree of red blood cells anisopokilocytosis varies and may be marked. The platelet and neutrophil counts are normal in most patients but occasional patients have an accompanying neutropenia or thrombocytopenia. In refractory anemia, the bone marrow blasts are less than 5 % ***(Brunning, 2001)***.

The number of erythroid precursors varies from marked erythroid hypoplasia to hyperplasia; erythroid hyperplasia is the predominant pattern. Ringed sidroblasts may be present but they make up less than 15 % of the nucleated red blood cells. Morphologic abnormalities usually are restricted to the erythroid series ***(Brunning, 2001)***.

Neutrophils and megakaryocytes are normal or increased. In approximately 10 % to 15 % of patients the marrow is hypocellular, and in some instances it may be markedly so, resembling aplastic anemia. Hypocellular bone marrow also may be observed in other types of MDS. This finding is more common in older individuals ***(Orazi et al., 1999)***.

2- Refractory anemia with ringed sideroblasts:

Anemia is always present with this type, and the hemoglobin level generally is in the range between 9 and 12 g/dl, lower levels may be present. The red blood cells may be dimorphic, with populations of both hypochromic and normochromic cells; in some instances the red blood cells are normochromic and macro- or normocytic. The platelets and neutrophils usually are normal in number and appearance. The bone marrow shows erythroid hyprerplasia, sometimes marked, with variable but usually minor degrees of dyserythropoiesis; in occasional patients, prominent dyserythropoiesis with megaloblastoid features is present ***(Brunning. 2001)*.**

In smears stained by Prussian blue, 15 % or more of the erythroid precursors are ringed sideroblasts. Granulopoiesis and megakaryocytopoiesis are essentially normal in most patients. The bone marrow biopsy shows a hypercellular marrow with prominent erythroid hyperplasia. Megakaryocytes and granulocytes appear to be morphologically and numerically normal. Sections stained for iron show numerous iron-laden macrophages ***(Brunning, 2001)***.

Sideroblastic anemia occurs in two forms: a true MDS with eventual evolution to acute myeloid leukemia or a higher grade MDS, and a type that probably results from some

inherited or acquired disturbance of iron metabolism. Patients with sidroblastic anemia should receive careful search for abnormalities in the granulocytic and megakaryocytic lineages; if either of these lineages is involved in addition to the erythroid series, the patient should be considered to have sidroblastic anemia with multilineage dysplasia ***(Kalaycio et al., 2006)*.**

3-Refractory cytopenia with multilineage dysplasia:

Some patients with MDS present with bicytopenia or pancytopenia with evidence of multilineage dysplasia without an increase in blasts or monocytes and no auer rods. The classification of these cases is problematic ***(Kalaycio et al., 2006)***. The term refractory cytopenia with multilineage dysplasia was introduced to recognize these patients ***(Rosati et al., 1996)*.**

Refractory cytopenia with multilineage dysplasia is characterizes by dysplasia of two or more myeloid cell lineages, frequently in all myeloid cell types, the degree of dysplasia may be marked. In some patients, one cell lineage, such as the eythroid precursors, is increased, with dysplastic changes, which is the predominant finding. The type and degree of dysplastic change vary substantially from patient to patient, and there is no unifying morphologic feature of this type of MDS ***(Brunning, 2001)***.

There is no or only a slight increase in myeloblasts in the bone marrow; if the percentage is less than 5%, blasts are not usually present in the peripheral blood. Essentially, the findings resemble RAEB without an increase in blasts. Bicytopenias is a common finding and frequently there is pancytopenia. Bone marrow biopsy may be particularly useful in recognizing the abnormalities in megakaryocytes, particularly small megakaryocytes and micromegakaryocytes with hypolobulated nuclei ***(Brunning, 2001)*.**

4-Refractory anemia with excess of blasts:

The defining criteria for refractory anemia with excess blasts (RAEB) are 5% to 19% blasts in the bone marrow. (Table 1) Based on data from the international MDS risk analysis workshop, which showed different survival data for patients with RAEB with 5% to 10% and those with 11% to 19% blasts in the marrow, RAEB is divided into RAEB-1 (5-10%) RAEB-2 **(**11–19%) ***(Brunning, 1999)***. Patients with blasts in the peripheral blood, but less than 11% in the bone marrow are classified as having RAEB-1.While patients with 11% to 19% blasts in the bone marrow or peripheral blood or less than 11% blasts in blood or bone marrow but blasts with Auer rods have RAEB-2. If the percentage of blasts in the peripheral blood exceeds the bone marrow blast percentage, the

blood blast percentage dictates the classification ***(Syemour and Estey, 1995 and Brunning, 1999)*.**

Anemia is present in virtually all patients, the red blood cells are normochromic and normo-or macrocytic. An increased incidence of anisopoikilocytosis is noted in a high percentage of cases, including macroovalocytes, nucleated red blood cells may be present. The majority of patients present with neutropenia and thrombocytopenia; pancytopenia is common. Neutrophil abnormalities include nuclear hyposegmentation, pseudo-Pelger-Huet nuclei, and hyperlobulation; abnormalities of granulation such as hypo granulation generally are present and frequently are marked.

The essential finding in the bone marrow is an increase in myeloblasts. In many patients, the increase in blasts is accompanied by an increase in promyelocytes. Ringed sidroblasts may be present and may exceed 15% of the nucleated red blood cells. The increase in blasts or the presence of auer rods, however, dictates the classification. Micromegakaryocytes characterized by small size and non lobulated nuclei may be numerous ***(Brunning, 2002)***.

The bone marrow trephine biopsy in the majority of patients is hypercellular with a panmyeloid hyperplasia; in 10% to 20% of patients, the bone marrow is normocellular or

hypocellular. The number of blasts in the sections usually corresponds to the smear or biopsy imprint differential. In some patients, foci of immature cells, blasts and promyelocytes, unrelated to an endosteal or perivascular location, may be noted.

The presence of three or more of these foci in a biopsy specimen, referred as abnormal localization of immature precursors, has been reported to be associated with an increased incidence of leukemic evolution ***(Tricot et al., 1998)***.

Abnormal megakaryocytes, particularly small megakaryocytes and micromegakaryocytes with nuclear hypolobulation, may be more apparent in the bone marrow sections than in smears; these cells are accentuated with the periodic acid-Schiff stain and immunohistochemical reactions with antimegakaryocytes antibodies ***(Orazi, etal.1999)***.

In patients with hypocellular bone marrows, the distinction of MDS from aplastic anemia may be aided by immunohistochemical reactions for CD34 and proliferating cell nuclear antigen; positive reactions for these antigens are more compatible with MDS than with aplastic anemia ***(Qrazi et al., 1999)*.**

5-Myelodysplastic syndrome unclassified:

Some patients with MDS present with morphologic feature that cannot be classified readily in any of the proposed MDS categories. These patients have no or minimal increase in blasts in the bone marrow and in most instances no blasts in the peripheral blood; in some a rare blast is identified in the peripheral blood. Dysplasia which may be slight or marked, affects a single lineage, in contrast to refractory cytopenia with multilineage dysplasia.

There may be cytopenia of one or more cell lines, including pancytopenia. Because of the lack of categorizing features from other types of MDS. These cases can be designated as MDS unclassified. Some observers might classify some of these cases descriptively according to the major hematologic finding, which is refractory anemia, refractory neutropenia, or refractory thrombocytopenia ***(Duell et al., 2006)*.**

The unclassified category also may be used for a type of MDS that presents with marked anemia with or without thrombocytopenia and neutropenia, and marked erythroid hyperplasia with marked dyserythropoiesis without an increase in blasts and no evident dysplasia in the neutrophils; the megakaryocytes may or may not show evidence of dysplasia (

Brunning, 2001).Such cases have some features of acute myeloid leukemia, M6B pure erythroid malignancy, but the numbers of proerythroblasts and basophilic erythroblasts are less than requisite ***(Brunning, 2001)*.**

The cases frequently are associated with clonal cytogenetic abnormalities involving chromosome 5 or 7 and may have an aggressive clinical course. The classification of this process is often difficult, because the distinction between acute myeloid leukemia M6 and MDS unclassified or refractory cytopenia with multilineage dysplasia is some what arbitrary. If the cytogenitic findings have an established relationship with MDS. This approach emphasizes the critical importance of clinical, morphological, and cytogentic assessment of MDSs ***(Brunning, 2001)*.**

Therapy related MDS:

Chemotherapy and radiotherapy to a lesser extent have been associated with the risk of developing MDS and AML, which begins from the start of therapy, with a peak incidence at 4 years, and with the risk beginning to disappear after 10 years, implying that a plateau is reached ***(Van Leenwen, 1996)*.**

The MDS phase usually precedes AML by 6 to 12 months. This type is best noted in patients receiving the alkylating agents mechlorethamine and procarbazine as part of