Cytogenetic Evaluation in 221 Untreated Patients with Myelodysplastic Syndrome

Tedavi Almamış 221 Miyelodisplastik Sendromlu Hastada Sitogenetik Değerlendirme

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ABSTRACT Objective: Myelodysplastic syndromes (MDS) are a heterogeneous group of malignant clonal hematopoietic stem cell disorders characterized by bone marrow failure, ineffective hematopoiesis, peripheral blood cytopenias, atypic cytological profile, increased apoptosis, and increased likelihood of evolution to acute myeloid leukemia (AML). Cytogenetic findings are major determinants in the diagnosis, classification, pathogenesis, prognosis, and treatment in patients with MDS. Cytogenetic analysis is a mandatory step in the full evaluation of a newly diagnosed patient. The aim of the present study was to retrospectively evaluate the cytogenetic findings of 221 MDS patients in İstanbul University Cerrahpaşa Medical Faculty, Medical Biology and Genetics Department. **Material and Methods:** Cytogenetic analyses of 221 patients (89 female, 132 male) were performed on bone marrow cells using a trypsin-Giemsa banding technique. Metaphase cells were obtained from short-term unstimulated cultures. When possible, at least 20 metaphases were analyzed and 10 of them were fully karyotyped. **Results:** Among the 221 patients, 122 had no karyotype anomalies (55.20%) and 99 (44.80%) had clonal cytogenetic abnormalities; with 46 (20.81%) having one, 19 (8.59%) having two and 34 (15.38%) having complex (≥3) abnormalities. According to the International Prognostic Scoring System (IPSS) cytogenetic categories, 130 (58.82%) patients presented with a good karyotype, 54 (24.44%) patients with intermediate karyotype and 37 (16.74%) patients with poor karyotype. **Conclusion:** Although some cases appear to have a normal karyotype, the technical failures such as inability to obtain sufficient analyzable metaphases may reduce the actual proportion of abnormal cases. The examination of 20 or more metaphases could further increase the sensitivity of cytogenetic analyses with clinical impact in individual cases by identifying additional abnormal clones or subclones.

Key Words: Myelodysplastic syndromes; cytogenetics

ÖZET Amaç: Miyelodisplastik sendromlar (MDS) kemik iliği yetmezliği, inefektif hematopoez, periferik dolaşım sitopenileri, atipik sitolojik profil, artmış apopitoz ve akut miyeloid lösemi (AML) gelişme olasılığının artması ile karakterize heterojen bir grup malin klonal hematopoetik kök hücre bozukluğudur. MDS'li hastaların tanı, sınıflama, patogenez, prognoz ve tedavisinde sitogenetik bulgular esas belirleyicilerdir. Yeni tanı konmuş hastada sitogenetik analiz mutlak gerekli olan bir aşamadır. Bu çalışmanın amacı, İstanbul Üniversitesi Cerrahpaşa Tıp Fakültesi, Tıbbi Biyoloji ve Genetik Anabilim Dalı'na başvuran 221 MDS hastasının sitogenetik bulgularını geriye dönük olarak analiz etmektir. **Gereç ve Yöntemler:** Trypsin-Giemsa bant tekniği ile 221 olgunun (89 kadın, 132 erkek) kemik iliği hücrelerinde sitogenetik analiz gerçekleştirildi. Metafaz hücreleri kısa dönemli stimülasyonsuz kültürlerle elde edildi. Mümkün olduğunda en az 20 metafaz analiz edildi ve bunların 10'u tümüyle karyotiplendi. **Bulgular:** Çalışmaya alınan 221 hastanın 122'sinde karyotip anomalisi (%55,20) yoktu, 99'unda (%44,80) klonal sitogenetik anormallik gözlendi; bunlardan 46'sında bir anomali (%20,81), 19'unda iki (%8,59) ve 34'ünde (%15,38) kompleks (≥3) anormallikler gözlendi. International Prognostic Scoring System (IPSS) sitogenetik kategorilerine göre 130 hastanın (%58,82) iyi, 54'ünün orta (%24,44) ve 37'sinin ise (%16,74) kötü karyotipe sahip olduğu belirlendi. **Sonuç:** Bazı hastaların normal bir karyotipe sahip olduğu görülmekle birlikte, yeterince analiz edilebilecek metafaz elde edememek gibi teknik zorluklar, anormal olguların oranını daha düşük algılamamıza neden olmuş olabilir. Yirmi veya daha fazla metafazın incelenmesi, sitogenetik analizin hassasiyetini artırarak belirli olgularda klinik önemi olan ek anormal klon ve alt klonların ortaya çıkarılmasını sağlayabilir.

Anahtar Kelimeler: Miyelodisplastik sendromlar; sitogenetik

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The myelodysplastic syndromes (MDS) are a heterogeneous group of malignant clonal hematopoietic stem cell disorders characterized by bone marrow failure, ineffective hematopoiesis, peripheral blood cytopenias, atypic cytological profile, increased apoptosis, and increased likelihood of evolution to acute myeloid leukemia (AML).[1-9] MDS can occur at any age, but most frequently develops in the elderly. The median age at diagnosis is 71 years and 72% of patients are 70 years or older.[6,10,11] Disease heterogeneity is reflected in the broad variability of morphological features in both peripheral blood and bone marrow, the variable clinical course (with median survival times ranging from a few months to several decades), and the presence of a great number of cytogenetic and molecular alterations in the abnormal cell clones.[7,8,12]

MDS is generally classified as "primary" (de novo), with no known mutagenic events, and "secondary" (treatment related), following a cytotoxic chemotherapy or radiotherapy. MDS are common in men than in women and in whites than in blacks. About 10% of the cases are secondary MDS.[6,8,11,13]

In 1982, French-American-British (FAB) cooperative group proposed a classification of five MDS groups based on cytomorphologic abnormalities and bone marrow blast count. For the last two decades, this classification has been the gold standard for patient follow-up/monitoring and clinical investigations. Various prognostic systems have been proposed in order to improve the ability to predict survival and progression in MDS patients, based on clinical variables including age, peripheral cytopenias, bone marrow blast count, lactate dehydrogenase level and cytogenetic pattern.[1,2,10,14-17]

In 1997, an International MDS Risk Analysis Workshop defined the International Prognostic Scoring System (IPSS) based on bone marrow blast percentage scored into four ranges (Low, Intermediate-1, Intermediate-2 and High), number of peripheral cytopenias and karyotype categorized in three groups (Poor, Intermediate and Good).[2,4,13,14,17,18]

In 2001, the World Health Organization (WHO) proposed a revision of the FAB morphological approach to improve homogeneity and discrimination between lower-risk MDS categories. This new classification is based on unilineage or multilineage dysplasia, blood count and cytogenetic features in MDS patients.[2,16,17,19]

Although the IPSS has been validated for the WHO classification, there is room for improvement. The WHO classification-based Prognostic Scoring System (WPSS) is based on WHO classification, cytogenetics and red blood cell transfusion requirement.[1,8,11,20]

Cytogenetic findings are major determinants in the diagnosis, classification, pathogenesis, prognosis, and treatment in patients with MDS. The WHO classification requires cytogenetic investigation for diagnosis; thus, cytogenetic analysis is a mandatory step in the full evaluation of a newly diagnosed patient. Classification of a hematological malignant disease can be a challenging undertaking, but it is crucial for the appropriate management of a patient. The detection of a cytogenetic abnormality may be useful in suspicious cases to establish the diagnosis of MDS. Clonal chromosomal anomalies are detected in 30-60% of primary MDS and 80% of secondary MDS cases. In general, MDS show a characteristic genetic profile with an overweighing of unbalanced abnormalities. Loss of genetic material in the form of deletions and monosomies are observed most frequently, whereas gain of genetic material with the appearance of total or partial trisomies are less frequent. Gain or loss of genetic material can also be the result of unbalanced translocations, which are frequently observed in MDS with multiple abnormalities.[2,3,6,10,12,13]

A prime molecular mechanism in MDS is assumed to be the loss or inactivation of tumor suppressor genes, while the activation of oncogenes seems to be less relevant in myelodysplasia. The most common aberrations in MDS are, deletion of the long arm of chromosome 5, monosomy of chromosome 7, deletion of the long arm of chromosome 7, trisomy 8, deletion of the long arm of chromo-

some 9, deletion of the short arm of chromosome 17, deletion of the long arm of chromosome 20 and monosomy of chromosome Y.[3,8,10,12,21,22]

In 1997, Greenberg published a collaborative multicentric international data set of 816 patients with primary MDS, which was the basis for the establishment of the IPSS.[14] The IPSS has placed cytogenetic abnormalities into 3 risk categories; good, intermediate and poor. The category of "good" includes a normal karyotype, deletion of 5q [del(5q)], del (20q), and loss of chromosome Y (-Y). The "poor" category includes a complex karyotype (≥3 abnormalities) as well as del (7q), -7 and any chromosome 7 abnormality present either as a single anomaly or in combination with other anomalies. All other abnormalities are included in the "intermediate" category.[2,12,13,14] Several reports established from large patient series have suggested that some of the less common cytogenetic abnormalities may play a very significant role in the clinical outcome of patients with MDS. Therefore, a reclassification of the IPSS cytogenetic risk groups has been proposed.[2,13] According to this reclassification, the cytogenetic abnormalities have been divided into four prognostic subgroups: *Good* (5q-, 12p-, 20q-, +21, -Y, 11q-, t(11q23), normal, 2 abnormalities including 5q-), *intermediate-I* (+1q, 3q21/q26- abnormalities, +8, t(7q), +19, -21, any other single, any other double), *intermediate-II* (-X, -7/7q-, two abnormalities including -7/7q-, complex constitution with 3 abnormalities), and *poor* (>3 abnormalities).[2,13,15,23]

The aim of the present study was to evaluate retrospectively the cytogenetic findings of 221 patients with MDS in İstanbul University Cerrahpaşa Medical Faculty, Medical Biology and Genetics Department.

MATERIAL AND METHODS

We retrieved data of 221 patients with suspected primary MDS who had undergone cytogenetic studies at İstanbul University Cerrahpaşa Medical Faculty, Medical Biology and Genetics Department from 1996 to 2010. The study population included 89 females and 132 males aged 1 month-88 years.

Cytogenetic analyses were performed on bone marrow cells using a trypsin-Giemsa banding technique. Metaphase cells were obtained from short-term unstimulated cultures. When possible, at least 20 metaphases were analyzed and 10 of them were fully karyotyped.

The International System for Human Cytogenetic Nomenclature ISCN 2005 criteria were used for chromosome identification and karyotype description. Karyotypes were defined as complex when they included three or more chromosomal abnormalities. According to the ISCN a clone was defined by the same structural aberration or chromosome gain in at least two metaphases, or loss of the same chromosome in at least three metaphases. Metaphase cells with additional clonal aberrations were considered subclones.[24,25]

RESULTS

Cytogenetic study was successfully carried out in 221 MDS patients. The age of patients ranged from 1 month to 88 years old with a median age of 55.39 years. The study population included 89 females and 132 males and the male to female ratio was 1.48:1, consistent with the well-known male predominance in MDS.

Among the 221 patients, 122 had no karyotype anomalies (55.20%) and 99 (44.80%) had clonal cytogenetic abnormalities; with 46 (20.81%) having one, 19 (8.59%) having two and 34 (15.38%) having complex (≥3) abnormalities. A systemic documentation of cytogenetic abnormalities (monosomies, trisomies, deletions of short or long arms, trisomies of short or long arms, additional material on short or long arms, translocations involving short or long arms, inversions or derivative chromosomes) was performed for every patient. The incidence of these chromosomal abnormalities were shown in Table 1.

Cytogenetic analyses revealed that chromosomes 6, 8, 10, 15, 21 and 22 had only numerical changes with gain or loss and the remaining chromosomes had both numerical and structural changes with gain or loss. Chromosomes 5, 7, 8, 18, 21 and Y were the most commonly involved in cytogenetic changes.

TABLE 1: Incidence of chromosome abnormalities in 221 MDS patients.

Chromosomal Abnormality	Isolated (n)	With one additional abnormality (n)	As a part of complex abnormality (n)	Total (n)
-X	1	1	5	7
inv(X)	1	0	0	1
t(X;?)	0	0	1	1
-Y	6	4	7	17
-1	0	0	1	1
+1	0	0	2	2
der(1)	1	0	0	1
1q+	0	1	0	1
t(1;1)(p36;q21)	1	0	0	1
t(1;2)(p36;p11)	1	0	0	1
t(1;3)(p10;q10)	0	1	0	1
t(1;7)(p12;p12)	1	0	0	1
-2-	0	1	0	1
+2	0	0	1	1
2p	1	0	1	2
-3	0	0	1	1
3q-	1	0	0	1
t(3;3)(q21;q26)	0	1	0	1
t(3;12)(p14;p12)	1	0	0	1
-4	0	0	5	5
+4	0	0	2	2
4p+	1	0	0	1
t(4;5)(p10;p10)	0	1	0	1
-5	0	3	7	10
+5	0	0	1	1
5q-	2	0	1	3
5q+	0	0	1	1
-6	0	1	6	7
+6	0	0	2	2
-7	3	3	8	14
7q+	0	1	0	1
-8	1	0	4	5
+8	3	3	4	10
-9	0	0	5	5
+9	0	0	1	1
9q-	1	0	0	1
9p+	1	0	0	1
t(9;22)(q34;q11)	2	0	0	2
t(9;10)(q22;q23)	0	0	2	2
inv(9)(p13q22)	0	1	0	1
-10	1	0	7	8
-11	0	1	7	8
+11	0	0	2	2
11q-	0	0	3	3
t(11;20)	1	0	0	1
-12	0	2	5	7
+12	1	0	1	2
12q-	0	0	1	1
t(12;15)(p12;q11)	0	0	1	1
t(12;21)(p12;p11)	0	1	0	1

TABLE 1: Continued

-13	0	0	3	3
+13	0	1	2	3
13q-	1	0	0	1
-14	1	0	6	7
t(14;15)	1	0	0	1
-15	0	2	2	4
+15	0	0	1	1
-16	1	1	8	10
16p+	0	0	1	1
inv(16)(p13;q22)	1	0	0	1
-17	1	0	9	10
+17	0	0	1	1
17p+	0	0	1	1
-18-	2	5	10	17
18p-	0	1	1	2
18q-	1	0	1	2
-19	0	0	10	10
+19	0	0	2	2
19q+	1	0	0	1
-20	1	0	8	9
20p-	1	0	0	1
20q-	0	1	1	2
-21	1	3	11	15
+21	1	1	1	3
-22	0	0	12	12
+22	0	1	0	1

inv: inversion
t: translocation

Of the 221 patients, 99 (44.80%) presented with cytogenetic abnormalities. According to the IPSS cytogenetic categories, 130 (58.82%) patients presented with a good karyotype, 54 (24.44%) patients with intermediate karyotype and 37 (16.74%) patients with poor karyotype. The frequency of IPSS cytogenetic subgroups was shown in Table 2.

DISCUSSION

In contrast to other hematologic diseases with homogenous genetic basis such as Chronic Myeloid Leukemia (CML), MDS show a profound heterogeneity not only on the morphologic and the clinial level but also on the genetic presentation.[7,12]

Even though MDS can occur at any age, most patients are old; 72% of the patients are diagnosed at age 70 or older. However, in areas of East Asia, it develops at ages almost 2 decades younger than in the rest of the world.[11] There are a few similar reports regarding younger age in MDS patients from China, Japan and India.[22,26] In our study, 28.96% of the patients were older than 70 years and the median age at diagnosis was 55.39.

MDS are more common in men than in women.[11,13,27] Consistenly, our study group included 89 females and 132 males and the male to female ratio was 1.48:1.

Various studies reported that the overall survival (OS) of male MDS patients was shorter than the female patients and the older patients had shorter survival. [1,14,17,28,29]

Cytogenetics is a significant component in diagnosis and assessment of prognosis for MDS pa-

TABLE 2: The frequency of IPSS cytogenetic subgroups.

IPSS subgroup	Number of patients (n)	(%)
Good	130	58.82
-Y	6	2.71
del(5q)	2	0.90
46,XX	54	24.44
46,XY	68	30.77
Intermediate	54	24.44
+8	3	1.36
Single abnormality	32	14.48
2 abnormalities	19	8.60
Poor	37	16.74
-7	3	1.36
7q+	1	0.45
Complex	33	14.93

Del, deletion; IPSS, International Prognostic Scoring System.

tients and also plays an important role in treatment selection as well as monitoring response to therapy.[13,27]

Numerous reports have demonstrated that karyotype abnormalities significantly affect OS and the risk of MDS/AML progression, even in patients undergoing intensive chemotherapies. Chromosomal abnormalities are the best predictors of outcome after intensive chemotherapy. In MDS/AML, patients with a normal karyotype tend to have a better response to chemotherapy.[5,7,30]

There is a great consensus in all publications on cytogenetic prognosis in MDS that complex abnormalities characterize an MDS subgroup with poor prognosis and a median survival time significantly below 1 year, although the threshold at which the number of abnormalities confers poor prognosis is controversial.[12]

Cytogenetic analysis of the bone marrow is indicated in MDS not only to detect characteristic chromosomal abnormalities, but also to assess clonal evolution.[8]

Since mosaic karyotypes are frequently found in MDS, fluorescence in situ hybridization (FISH) represents an advance in those cases; particularly interphase fluorescence in situ hybridization is a useful technique in patients with no analyzable metaphases.[4,31,32] However, several studies have compared FISH and conventional cytogenetic analysis at specific times during the development of the disease and most of them have established only a small advantage of FISH to detect chromosomal abnormalities; because, classical FISH is a targeted method, which allows only to identify the changes that are indicated by strictly defined molecular probes.[3,21,33] FISH was suggested to be used in selected cases where insufficient numbers of metaphases are available for Standard G-banding.[4,8,21,32]

In our study, clonal chromosome abnormalities had an incidence of 44.80%. Deletions within the long arm of chromosome 5 are the most frequent changes in MDS accounting for roughly 30% of abnormal cases. The deletions can have variable size; however, the common deleted region always spans the chromosome band 5q31. Some patients with isolated 5q- are categorized by the WHO classification as the "5q- syndrome". Such patients typically have a low frequency of progression to AML (10%) and favorable survival compared with other MDS subgroups. However, the patients with 5q- and additional cytogenetic abnormalities have a poor prognosis.[7,8,10,12,34] In our series we had 15 patients with abnormalities in chromosome 5. Only 2 of them were isolated 5q- and there was one 5q- as part of a complex karyotype. In addition, 10 patients presented with -5, seven of them as part of a complex karyotype and three of them with an additional abnormality. The ratio of 5q-/-5 abnormalities was 13.13% among the abnormal cases (Table 1).

-7/7q- is observed as the sole abnormality in approximately 5% of primary MDS cases and 55% of secondary MDS cases. Monosomy 7/7q deletion has been associated with a poor prognosis in terms of either short survival from diagnosis or leukemic evolution, thus, the IPSS has included these defects in the poor cytogenetic category. However, there are studies supporting the proposal to reclassify interstitial deletion of 7q to the intermediate cytogenetic risk categories.[5,10,13,14] In our study, we detected 14 cases with monosomy 7. Three of them were isolated, another three were -7 with an addi-

tional anomaly and eight were -7 as part of a complex karyotype. The ratio of monosomy 7 was 14.14% among the abnormal cases (Table 1).

The incidence of chromosome 8 gain in MDS is ~10% and sole +8 is categorized in the intermediate cytogenetic subgroup according to the IPSS. Trisomy 8 is the most common primary abnormality of MDS patients in several Asian countries. Some reports suggested that +8 should be included in poor cytogenetic risk group as some patients with +8 tended to progress rapidly to acute leukemia and have a short survival. However, there are also studies where no significant difference in median survival has been observed between patients with +8 as a single abnormality and patients with +8 associated with another cytogenetic abnormality.[7,10,13,35-38] In our study, there were 10 patients with trisomy 8. In three of them, trisomy 8 was the only abnormality, in another three there was an additional abnormality and in four, a complex karyotype was detected. The ratio of trisomy 8 was 10.10% among the abnormal cases (Table 1).

Deletion of the long arm of chromosome 20 is a common recurring abnormality in malignant myeloid disorders. This abnormality is seen in approximately 5% of primary MDS cases and 7% of secondary MDS cases. Cytogenetic analysis of the deleted chromosome 20 homologues reveal that the deletions are variable in size; the majority of them are large, mostly with loss of 20q. According to IPSS, 20q- is included in the good cytogenetic subgroup. Deletion of the long arm of chromosome 20 is suggested to be associated with a favorable outcome when noted as a sole abnormality, but with less favourable prognosis in the setting of a complex karyotype. This phenomenon is similar to that observed for the 5q- in MDS (discussed above).[5,10,14,27] In our series we found 12 patients with abnormalities involving chromosome 20. Only one of them was isolated -20 and eight were -20 as part of a complex karyotype. One patient presented with 20q- with an additional abnormality and one patient had 20q- as part of a complex karyotype. The ratio of 20q-/-20 abnormalities was 11.11% among the abnormal cases (Table 1).

Loss of chromosome Y can represent a normal age-related process or an MDS clone. Even when it represents an abnormal clone, it is uncertain whether the -Y is associated with disease pathogenesis. -Y as a sole cytogenetic abnormality is associated with a favorable outcome in MDS and is included in the good cytogenetic subgroup according to the IPSS.[10,13,14] In our series there were 17 cases with loss of chromosome Y. Six of them were isolated, four were accompanied with an additional anomaly and seven were part of a complex karytoype. -Y accounted for 17.17% of the abnormal cases in our study (Table 1).

The most frequent cytogenetic changes in our series were chromosome 18 abnormalities. There were 21 patients with chromosme 18 anomalies, accounting for 21.21% of the cases with abnormal karyotype. There was one 18p- with an additional abnormality and one 18p- as part of a complex karyotype. One of the two 18q- cases presented as a single abnormality and the other 18q- was part of a complex karyotype. Two of the monosomy 18 cases were single abnormalities, while five of them were accompanied with an additional anomaly, and ten were part of a complex karyotype (Table 1). In a series of 2124 patients, Haase et al. also reported -18/18q- as the fourth most frequent abnormality.[2]

The second most frequent cytogenetic abnormality in our study involved chromosome 21. Eighteen patients had chromosome 21 anomalies, accounting for 18.18% of the cases with abnormal karyotype. One of the fifteen monosomy 21 cases was an isolated abnormality, three were accompanied with an additional abnormality and eleven were part of a complex karyotype. One of the three +21 cases presented as a single abnormality, one had an additional abnormality and the remaining +21 was part of a complex karyotype. Chromosome 21 anomalies have been reported in a number of reports and are predominantly observed in complex karyotypes.[2,13,22]

CONCLUSION

In summary, cytogenetic findings have an established role in the diagnosis and assessment of prognosis of MDS and are emerging as an important

factor in treatment selection and monitoring response to therapy. MDS show a profound heterogeneity, not only on the morphologic and clinical level but also on the genetic presentation. In addition, while some of the cases appear to have a normal karyotype, technical failures such as the inability to obtain sufficient analyzable metaphases, may reduce the actual proportion of abnormal cases. In our study, we analyzed at least 20 metaphases for each case and found that all chromosomes had undergone either numerical or structural changes. We suggest that the examination of 20 or more metaphases could further increase the sensitivity of cytogenetic analysis with clinical impact in individual cases by identifying additional abnormal clones or subclones.

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