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Cytogenetic Anomalies in Multiple Myeloma Patients: A Single Center Study

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Abstract

Conventional karyotyping in the patients with Multiple myeloma (MM) is very important because the detected chromosomal abnormalities have diagnostic and prognostic value. In this retrospective study we aim to evaluate cytogenetic abnormalities in 133 MM patients whose diagnosis was established at the Hematology Department of Hacettepe University. The bone marrow samples were treated with trypsin and stained with Giemsa (GTG banding). For each patient, 20 metaphases were examined and karyotypes were formed. Cytogenetic results of the patient's bone marrow samples were obtained in 114 patients. Of 114 examined karyotypes, 80 patients had normal karyotype while 34 patients had abnormal karyotypes. Both numerical and structural chromosomal anomalies were detected in patients with abnormal karyotype. The most frequent numerical and structural anomalies were detected in chromosomes 1, 9, 16 and 13. The anomalies we found in our patient group were consistent with the literature.

Keywords

Multiple myeloma, cytogenetic, chromosomal abnormalities

Introduction

Multiple myeloma which is a highly heterogenous clonal disease of plasma cells (Kapoor & Rajkumar, 2011; Avet-Loiseau & Facon , 2018) There is a clinical heterogeneity among myeloma patients due to the underlying genetic heterogeneity. Genetic heterogeneity can be observed at chromosomal level as well. Multiple myeloma patients exhibit both numerical and structural chromosomal abnormalities (Nemec et al., 2012; Carrasco et al., 2006). Generally, patients may have hyperdiploid karyotypes or non-hyperdiploid karyotypes. In the hyperdiploid karyotypes, trisomies of 3, 5, 7, 9, 11, 15, 19, 21 chromosomes and fewer structural aberrations are seen. On the other hand, in non-hyperdiploid karyotypes there may exist some translocations which involve the

immunoglobulin heavy chain (IGH) locus. The most frequently observed immunoglobulin heavy chain translocations are t(14;16)(q32;q23), t(11;14)(q13;q32) and t(4;14)(p16;q32) (Ooi et al. 2016) . Conventional karyotyping is significant in MM as ploidy number has prognostic value. Hyperdiploid karyotpe signifies good prognosis while hypodiploid karyotype signifies the bad one. The chromosome 13 abnormalities, its deletion signifies bad prognosis. Anomalies of 14q32 region which has a various translocations partners in MM could be high risk for MM depending on the translocation partner (Rajan & Rajkumar , 2015; Levin et al., 2018; Lonial, 2010; San-Miguel et al., 2009; Zhan et al., 2006). Deletion of 1p and amplification of 1q are the most common cytogenetic abnormalities seen in MM patients. They are associated with bad prognosis and especially anomaly of 1q is taken as genomically unstable tumor marker (Sawyer , 2011; Szalat & Munshi, 2015; Brioli et al.,2014; Cagnetta et al.,2015).

In this retrospective study, cytogenetic data of MM patients are evaluated. We aimed to compare chromosomal anomalies, found in MM patients data with the literature findings.

Materials and methods

Patients

A total of 133 MM patients who were referred to Hacettepe University Department of Basic Oncology cytogenetic laboratuvary for routine karyotype analysis between 2008 and 2015 years were included in this study. Of the 133 patients with MM, 74 (55.6%) were male and 59 (44.3%) were female (Figure 1).

Method

Cytogenetic analysis of MM patients was performed on metaphase cells derived from 24-h unstimulated bone marrow aspirate cultures. All the procedures were performed in accordance with the Helsinki declaration and approved by the local ethics committee (Approval no: GO 15/544-10) to collect human bone marrow for diagnosis. For complete karyotyping of samples a minimum of 20 metaphases were analyzed. Briefly, the cells were synchronized using uridine, fluorodeoxyuridine and thymidine. Colcemid was added to culture (0.05 g/ml) for half an hour before harvesting. After 30 min incubation in hypotonic solution (0.075 M KCl), the cells were fixed with Carnoy's solution (3 parts methanol to 1 part glacial acetic acid). After one night aging at 65 oC, the samples were treated with trypsin and stained with Giemsa (GTG banding). The preparations ready for analysis were examined using the image analysis system (Metasistem/Germany). The karyotypes were interpretted according to the 2015 International System for Human Cytogenetic Nomenclature (Haffer LG et al.,2013).

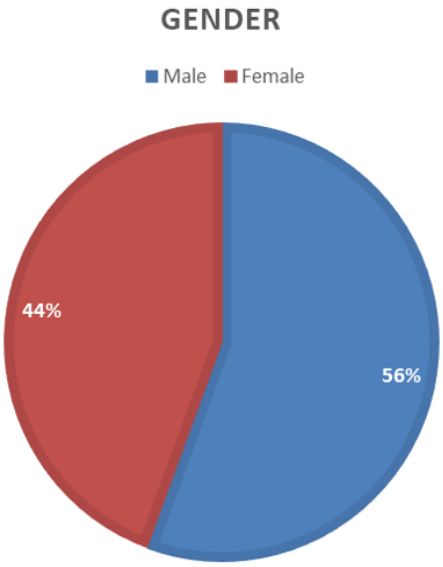

Figure 1 Graph includes 133 patients suffering from Multiple Myeloma. Of these, 55.6% are male and 44.3% are female.

Results and discussion

Cytogenetic analyses of the bone marrow samples were not successful in 19 (14.2%) patients while in 114 (87.2%) patients complete karyotype was obtained. Conventional cytogenetic results of these 114 patients showed that 80 patients had normal karyotypes while 34 patients had abnormal karyotypes (Figure 2).

As demonstrated in table 1, both numerical and structural chromosomal anomalies were detected in patients with abnormal karyotype.

Hyperdiploid karyotypes were found in 13 (38.23%) and non-hyperdiploid karyotypes (hypodiploid, pseudodiploid and near-tetraploid) were found in 18

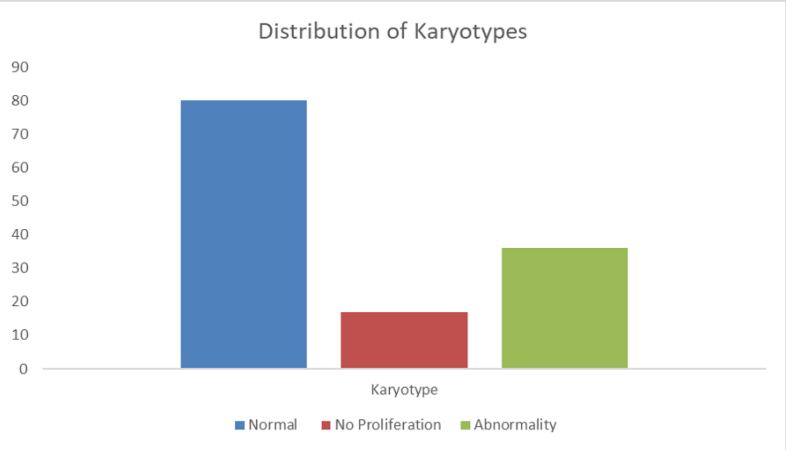

Figure 2. Among 133 Multiple Myeloma cases, 80 patients with normal karyotpe (blue), 34 patients with abnormal karyotype (green) were observed. 19 patients had no karyotype results (red).

Table 1. Karyotypes of the Multiple Myeloma Patients

Patient No	Karyotypes
1	49,XY,+4,+7,+9,+20,-2[7]/46,XY[43]
2	47-57,XY[9]/46,XY[31]
3	59,XY,+2,+3,+4,+5,+7,+8,+9,+11,+11,+15,+19,+21,+21[5]/46,XY/[45]
4	46,XY,-9,-11,-14,-16,+mar1,+mar2,+mar3,+mar[46]/46,XY[14]
5	46-50,XX,-8,-9,+14,+15,-16,+18,+18,-19,+19,-22,+22,+mar[12]/46,XX[8]
6	49-55,XX,+X,del(1)(p31),-1,-4,+5,-6,+7,+8,-11,+12,-12,-13,-14,+17,-17,+18,+19,+20,+21,+22,+mar1,+mar2;+mar3,+mar4,+mar5,+mar6,+mar7,+mar8[52]/46,XX/[38]
7	53,X,-X,-2,+3,+5,-8,+9,+11,+15,+19,+21,+mar1,+mar2,+mar3[4]/46,XX[46]
8	46,XX,+3,-4,-4,-7,der(8),-9,del(12)(p13),-14,-14,+15,-16,-19,-22,+mar1,+mar2,+mar3,+mar4,+mar5,+mar6,+mar7[23]/46,XX[77]
9	46,XY,der(1)[20]
10	46,XY,der(9)[20]
11	45,X,-Y,[3]/46,XY[47]
12	50,X,-X,+del(1)(p35p21),+5,+5,-11,-16,+19,+mar1,+mar2,+mar3[8]/46,XX[52]
13	60,XX,+3,+4,+5,+6,+7,+9,+11,+11,+15,+16,+19,+19,+21,+21[15]/46,XX[40]
14	45,X,-Y,[4]/46,XY[46]
15	46,XY,der(16)[20]
16	44,X,-X,-1,-8,-8,del(9)(p24p21),-13,+mar1,+mar2,+mar3[22]/46,XX[23]
17	45,XY,i(1)(q10),-2,-13,-14,del(16)(q24),+mar1,+mar2[15]/45,XY,i(1)(q10),-2,-13,-14,-14,del(16)(q24),+mar2,+mar3,+mar4,[10]/84-98,[4n],XY,i(1)(q10),-2,-13,-13,-14,-14,del(16)(q24)x2,+mar1,+mar2,+mar5[40]/46,XY[5]
18	46,XY,dic(1;21)(p11;p11),del(3)(q25q29),del(6)(q24q26),t(11;14)(q13;q32),del(13)(q14q21)[20]
19	44-46,XY,-3,-5,del(7)(q31q36),-9,-10,-12,-14,-15,-17,-18,+mar1,+mar2,+mar3,+mar4,+mar5[18]/46,XY[2]
20	45,X,-Y[14]/46,XY[56]
21	50-53,-X,-Y,del(3)(q21q26),+5,+9,+11,-13,der(14),+15,-16,+18,-22,+mar1,+mar2,+mar3,+mar4,+mar5,+mar6,+mar7[20]
22	46,XX,del(11)(q23q25),-18,+mar1[15]/46,XX[5]
23	90,XY,+X,der(1)x2,del(1)(p13p16)x4,+2,+3,+3,+5,+6,+7,+8,+8,+9,+10,+10,+11,+12,+13,+13,+14,+15,+16x4,+17,+18,+19,+19,+20 x4,+21,+mar1,+mar2,+mar3,+mar4,+mar5,+mar6,+mar7[2]/46,XY[18]
24	46,XX,der(3),t(11;14)(q13q32)[16]/58-81[4]
25	68-82,XY,+X,-Y,+1,+1,+2,+2,+3,+3,+4,+5,+6,+6,+7,+7,+8,+8,+9,+10,+11,+11,+12,+12,+13,+13,+der(14),+15,+15,+16, +18,+18,+19,+20,-21,+22,+mar1,+mar2,+mar3,+mar4[10]/46,XY[70]
26	46,XY,del(1)(q31q36),-12,-13,-17,+19,+mar1,+mar2[9]/46,XY[11]
27	40-42,XY,-1,-5,-6,-7,-13,-15,-16,-17,-20,-20,-21,+mar1,+mar2,+mar3,+mar4,+mar5,+mar6[35]/46,XY[15]
28	45,XX,-2,-8,-11,-16,-16,+mar1,+mar2,+mar3,+mar4[18]/46,XX[2]
29	46,XX,-5,-12,-22,+mar1,+mar2,+mar3[3]/46,XX,-2,-5,-12,-22,+mar1,+mar2,+mar3,+mar4[3]/46,XX[14]
30	44,X,-Y,del(1)(q32q43),-11,-14,-17,+mar1,+mar2(2}/46,XY[18]
31	47-95,X,-X,-3,-4,-4,-8,-9,-11,-11,-13,+21,+mar1,+mar2,+mar3,+mar4,+mar5,+mar6,+mar7,+mar8[13]/46,XX[7]
32	46-48,XY,+Y,-1,-2,-3,-11,+20,+mar1,+mar2,+mar3,+mar4[7]/46,XY[13]
33	58-59,XY,+3,+5,+5,+6,+9,+9,+11,+11,+15,+17,+19,+20,+21[20]
34	44,XY,der(1),+del(1)(p13p36),+t(9;9)(p22;p24),-13,dert(14),-15,-16,-22,+mar1[17]/46,XY[3]

(52.94%) of 34 patients with abnormal karyotypes. Among the patients with hyperdiploid karyotypes, 5 had only trisomies and monosomies. The other 4 hyperdiploid karyotypes had marker chromosomes beside trisomies and monosomies and 4 patients with hyperdiploid karyotypes had deletion of 1p and deletion of 3q in addition to trisomies, monosomies and marker chromosomes. Among 15 non-hyperdiploid karyotypes 5 of them were pseudodiploid and 10 of them were hypodiploid karyotypes. Pseudodiploid karyotypes were complex karyotypes which had more than three chromosomal abnormalities. These complex pseudokaryotypes had structural and numerical abnormalities. Monosomies and trisomies of many chromosomes were found in these karyotypes. Monosomies of chromosomes 9, 14, 16, and 12 were common in all pseudokaryotypes. Additionally, trisomies of chromosomes 3, 15 and 9 were found. Beside these numerical deviations, various structural anomalies were determined in pseudodiploid karyotypes as derivate 8, deleletion of chromosome 12p13, deletion of chromosomes 11q23q25 and deleletion of chromosome 1q31q36. Marker chromosomes were also determined in all pseudodiploid karyotypes. In hypodiploid karyotypes having chromosome numbers between 40-45, various monosomies were detected. Among them, monosomies of chromosomes 1, 5, 13, 14, 15 and 17 were particularly common in most hypodiploid karyotypes. Del(16)(q24), del(7)(q31q36), del(1)(p13p36), del(9)(p24p21), derivate 1, derivate 14, i(1)(q10), t(9;9)(p22;p24) were also seen in these hypodiploid karyotypes as structural abnormalities. In 4 patients with abnormal karyotypes only structural abnormalities were detected. In three of them derivate 1,9 and 16 chromosomes were seen as sole genetic abnormalities in all metaphases. Also, dic(1;21) del(13), del(6) and t(11;14) were detected in one patient. When we retrospectively look at the cytogenetic results of 133 MM patients, it was observed that 114 of the patients were karyotyped. While normal karyotype was determined in 80 of these 114 patients, abnormal karyotype was found in 34 patients. No results were obtained in 19 patients. Of the 34 patients with abnormal karyotype, 28 had complex karyotype which means karyotypes were carrying 3 or more chromosomal anomalies. There were also diploid karyotypes with anomalies. Loss of chromosomes Y was detected in 2 of these non-complex karyotypes. In the other 3 metaphases with non-complex karyotype, derivate 1, derivate 9 and derivate 16 were found.

Both numerical and structural anomalies were determined in complex karyotypes. Numerical and structural anomalies of chromosomes 1, 9, 16 and 13 were detected most frequently among these complex karyotypes (Figure 3). Chromosome 1 anomalies identified can be summarized as; derivate 1,

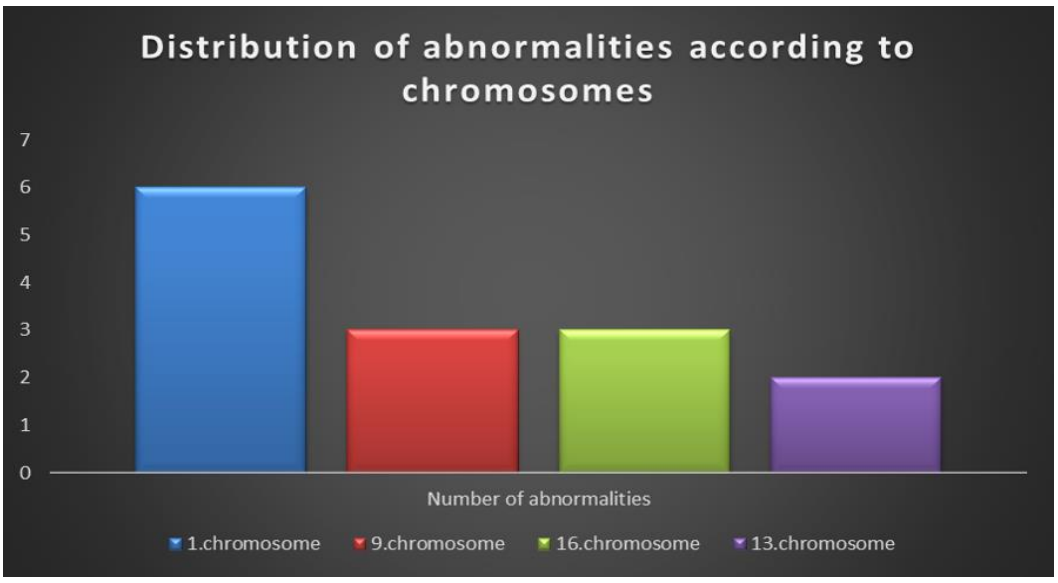

Figure 3. Numerical and structural anomalies of chromosomes 1, 9, 16 and 13 were detected most frequently among Multilple Myeloma patient

t(1;21)(p11;p11), del(1)(p) and del(1)(q). Chromosome 1 anomalies are the most common anomalies in MM. Anomalies associated with chromosome 1 in MM are deletion in p arm and increase in q arm (Marzin et al., 2006; Chang et al., 2010). In a study of Wu et al. 2016 chromosome 1 and chromosome 13 abnormalities were the most frequent abnormalities in MM patients. In our study we observed correlated results, displaying chromosome 1 and chromosome 13 anomalies as the most frequent ones. Trisomies of chromosomes 5, 9, 11, 13, 15, 19 and 21 and monosomies of chromosomes 11, 13, 14, 16 and 17 were detected most frequently in patients with hyperdiploid karyotype. On the other hand, chromosomes 1, 4, 5, 13, 14, 15 and 17 monosomies were found most frequently in patients with hypodiploid karyotype. When we examined the karyotypes in our study group, we observed that structural anomalies were more frequent in hypodiploid karyotypes rather than hyperdiploid karyotypes. In hyperdiploid karyotypes trisomies of various chromosomes and marker chromosomes also, del(1)(p31), del(3)(q21q26), derive 14 and +del(1)(p35p21) were detected. This last anomaly leads to the 1q amplification seen frequently in MM cases. According to our results, although hypodiploid karyotypes have more structural anomalies than hyperdiploid karyotypes, anomalies seen in both groups were common anomalies in MM. For example patients with hypodiploid karyotype have chromosome 1 anomalies such as i(1)(q10), del(1p) like patients with hyperdiploid karyotypes.

In one patient with pseudodiploid karyotype, dic(1;21)(p11;p11) was found. Translocation between chromosomes 1 and 21 in these break points was reported for the first time by Okay M et al., 2019. This patient also had t(11;14)(q13;q32) which is one of the most frequent translocation seen in MM.

Conclusions

Prediction of survival and management of risk classification in MM patients is crucial. Cytogenetic data are important prognostic factors in MM. Therefore, detection of cytogenetic anomalies is very important in MM patients. In this study, we examined the karyotypes of MM patients retrospectively and found that the anomalies we found were compatible with the literature. As in other hematological malignancies, MM needs more genetic information for better understanding the pathogenesis of the disease. Therefore, it is thought that more cytogenetic data of MM patients will serve to shed light on new treatment options.

Conflict of interest

Authors declare no conflict of interest.

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