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Analysis Of *TET-2, ASXL1, IDH1, IDH2* Gene Polymorphisms and The Correlation with Clinical Parameters in Patients With Myelodysplastic Syndrome

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Abstract

Research Article

Myelodysplastic syndrome (MDS) represent heterogeneous group of disorders with a variety of features including peripheral cytopenia, characteristic morphological findings and cytogenetic abnormalities in bone marrow. Some genes are reported to be involved in the pathogenesis of MDS such as IDH1-2, TET2 and *ASXL1*. Thus, identifying the recent mutations of these genes and genotyping the patients for these mutations might have clinical impact in MDS. We aimed to determine the genotype distribution and allele frequency of selected genes in MDS cases. Total 100 patients were genotyped for 5 mutations. For DNA isolation was peripheral blood was used. The cases were genotyped with sequence detection Systems software for allelic discrimination for each mutation. Age at diagnosis, gender, WHO classification, IPSS group, diagnosis (WBC-Hb-PLT) compared with polymorphisms (wild-type, heterozygous, homozygous mutants) were not statistically significant differences. Only *ASXL1* heterozygous group median age at diagnosis was significantly higher than homozygous (p=0,048). No significant association with the prognostic impact any of the polymorphisms. *TET2* heterozygous polymorphic group of azacitidine treatment response was significantly higher compared to the wild type. (p=0,042). Our study is the starting point for genotyping these polymorphisms that might have clinical guidance for MDS.

INTRODUCTION

MDS is a clinically and cytogenetically heterogeneous clonal disease, characterized by ineffective hematopoiesis and cytopenias in peripheral blood, with the risk of conversion to Acute Myeloid Leukemia (AML). MDS often affects the elderly; 80% of the cases were diagnosed after 60 years of age and the median age of diagnosis is 76[1]. The frequency of all age groups in the United States is 3-4 per 100,000 [2]. The incidence of age above 70 goes up to 22-45 in 100,000 and increases with age[2]. In the MDS classification, the World Health Organization (WHO) 2008 classification is used.

WHO classifies Refractory anemia with refractory cytopenia and single serial dysplasia [(Refractory thrombocytopenia (RT), Refractory Anemia (RA), Refractory Neutropenia (RN)], Refractory anemia with ring sideroblast (RARS), Refractory cytopenia with multiple serial dysplasia (RCMD), Refractory anemia 1-2 with refractory cytopenia blast increase, isolated del 5q and unclassified MDS (MDS-U).

Prognostic scoring is the most important determinant in the choice of treatment in patients with MDS. In 1997, the International Prognostic Scoring System (IPSS) and the WHO Prognostic Scoring System (WPSS) were established. In IPSS, karyotype (good-moderate-bad), cytopenia and bone marrow blast rates are used. WPSS is a new risk classification system that takes into account the need for transfusion of patients, whereas IPSS is more widely used. In 2012, the revised scoring system (R-IPSS-Revised International Prognostic Scoring System), which was created by the discovery of new molecular markers, was established, but has not yet been used in the clinical setting [3]. Patients are divided into groups according to risk score and their treatment is planned.

The pathogenesis of MDS has not been elucidated. About 50% of MDS patients have karyotype abnormality. SNP sequence-based karyotyping plays a role in the detection of small imbalances in the genome and segmental uniparental dysomy. A large number of mutations and chromosomal aberrations have been reported in the literature. Mutated genes are generally genes belonging to 4 different functional groups. These gene groups perform cytokine signal, DNA methylation, histone modification and spleosome functions in the cell. Abnormal differentiation in MDS is caused by loss of DNA

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methylation. TET2 and IDH 1-2 involve in methylation and ASLX1 plays a role in histone methylation control. TET2 is a gene located at 4q24 in the genome. It encodes alpha ketoglutarate (aKG) and Fe (II) -dependent hydroxylase enzymes, converting 5-methylcytosine (5mC) to 5-hydroxymethylcitosine (5hmC).

TET2 mutation is seen in 20% of patients with MDS and 30-50% in Chronic Myelomonositer Leukemia (CMML). There are studies showing that the detection of TET2 mutation, which is common in MDS patients, is associated with a good prognosis independent of IPSS [4].

Itzykson et al. (2011) in their study showed that patients with TET2 mutation respond to AZA treatment better than mutation negative patients [5]. Epigenetic abnormalities in MDS may occur earlier, for instance, the TET2-ASXL1 mutation can be seen years before transformation into AML.

However, TET2 mutation has also been reported in elderly individuals with hematopoiesis but without significant MDS [5]. ASXL1 interacts with histone modifiers and is located at 20q11.21 in the genome. ASXL1 was detected in 40% of CMML in 10-20% of patients [6]. ASXL1 is the most well-defined and the most common mutation after TET2 and is a poor prognostic indicator in low-risk patients.

Thol et al. (2011) showed that the conversion time to AML was shorter in such patients. IDH is the nicotinamide adenine dinucleotide (NADP+)-dependent crebs cycle enzyme that converts the isositrate to α-KG. In the genome, IDH-1 is located in 2q33.3 and IDH2 is in 15q26.1. IDH1 and 2 produce the α-ketoglutarate (α-KG) required for TET2 and the mutation is detected in <10% (4-12%) of patients. It has been shown that 2-hydroxyglutarate (2-HG) produced by mutated IDH1-2 proteins inhibit α-KG-dependent enzymes such as TET2 and this results in DNA hypermethylation [7].

In a meta-analysis to evaluate the effect of IDH mutations on prognosis, it has been shown that this mutation affects overall survival in the mid-1 MDS group [7]. New epigenetic markers may be included in the classification system used for MDS and may be useful in determining prognosis. In addition, advanced studies can be used to improve treatments that affect epigenetic regulators. AZA and Decitabine are the drugs prescribed in clinical use and have been shown to be effective [8].

In literature, studies evaluating polymorphisms of methylation genes in MDS are not abundant. In our study, we aimed to determine the TET2 rs763480, ASXL1 rs2208131, IDH1 rs11554137, IDH2 rs267606870 gene polymorphisms in MDS patients and to evaluate the relationship of these polymorphisms with the clinic.

MATERIALS and METHODS

Collection of patient data and samples

The study was conducted between February and August 2015 in the Hematology Outpatient Clinic of Ege University Faculty of Medicine. The patients were followed up or newly diagnosed with MDS, aged 18 and more, and received supportive therapy or all treatment modalities except classical chemotherapy.

In the policlinic follow-up file of the patients, hemogram, biochemistry (LDH-Ferritin-Albumin-Globulin) values, bone marrow aspiration and pathology (blast rate, cellularity, reticular fiber grade) results, karyotype and FISH results, EPO levels were recorded and the treatments also the transfusion frequencies were determined.

Each participant agreed to participate in the study by signing the Volunteer Consent Form. A total of 2 tube blood samples were collected in 3 ml of EDTA tubes. Peripheral blood samples were taken approximately 1 month after the last transfusion and in patients requiring more frequent transfusions immediately before transfusion. Our study protocol was approved by the Research Ethics Committee of Ege University Faculty of Medicine on 02.03.2015 and numbered 15-2.1/13.

DNA isolation from peripheral blood samples

DNA was isolated from peripheral blood samples taken from EDTA tubes according to the commercial kit protocol (Roche MagNA Pure 24 System).

Following DNA isolation, quantitation of each DNA was measured with NanoDrop and appropriate samples were included in the study.

Real Time PCR

Real-time PCR processing for SNP assays was performed with isolated DNA. These processes were performed on the ABI 7500 Fast real-time PCR instrument using TaqMan SNP genotyping assay (AppliedBiosystems, Foster City, CA) with FAM (specific for Allel 1) and VIC (specific for Allel 2) with TaqMan ® MGB probes.

After PCR was performed, Sequence Detection Systems (SDS 2.0) was used to automatically collect data for allelic discrimination. With this software, genotyping was performed according to the increase of fluorescence signal in each sample and the findings were interpreted according to the manufacturer's manual.

The cases were evaluated as polymorphic (homozygous mutant) when there was an increase in fluorescence in FAM, wild type when there was an increase in VIC, and heterozygote genotype when there was an increase in both.

Statistical analysis

The distribution tests (Saphiro and / or Kolmogorov-Smirnov test) determined whether the distribution of numerical parameters was normal. Continuous variables with normal distribution were given as mean ± standard deviation, and variables which were not distributed according to normal distribution were given as median / range. Mann-Whitney U test and Kruskal-Wallis test were used in comparison of the two groups. The chi-square test was accepted as $p>0.05$ equilibrium and it was used for comparison of qualitative data, to analyze the distribution of alleles to the genotypes, to check the compatibility of this distribution with the expected values (Hardy-Weinberg equilibrium) and to compare genotypes with other parameters.

Overall survival was compared using the Kaplan Mayer and the long rank test, from the diagnosis of the disease to the time until death or the last control time. Progression-free survival was calculated from diagnosis to leukemic transformation or death. The results were evaluated at significance level $p<0.05$ and 95% confidence interval. Statistical Package of Social Sciences, SPSS for Windows, Version 20.0, Chicago, IC, USA (SPSS 20) were used for other statistical analysis.

RESULTS

Demografic data

In this study, out of 100 patients 55 were female and 45 were male. The mean age at diagnosis was 62.67 ± 14.218 years, while the median patient age was 65 years (youngest 24 and oldest 89 years old). The median diagnosis WBC was 4.630 10^9 / L (0.809-18.1), Hb 9.9 g / dl (4-14.7), PLT 150, 5 10^9 / L (12-769). Median diagnosis Ferritin; 90.55 (2,3-4438) ng/mL, median diagnosis LDH; 278 ± 130,85 (U/L), albumin; 4,251 ± 0,61 g / dL and globulin were found to be 2,771 ± 0,51 g / dL (Table 1).

Table 1: Demographic Characteristics of Patients According to Blood Values

	Min	Max.	Mean	Median	SS
Age	24	89	62,67	65	14,218
WBC 10^9/L	0,809	18,1	5,382	4,63	3,239
Hb (g/dl)	4	14,7	9,78	9,9	2,1208
Ferritin (ng/mL)	2,3	4438	325,01	90,55	609,51
PLT 10^9/L	12	769	193,723	150,5	139,902
LDH (U/L)	115	678	278	240	130,85
Albumin (g/dL)	0,5	5,5	4,251	4,3	0,61
Globulin (g/dL)	1,8	4,5	2,771	2,700	0,51

Cytogenetic karyotype results

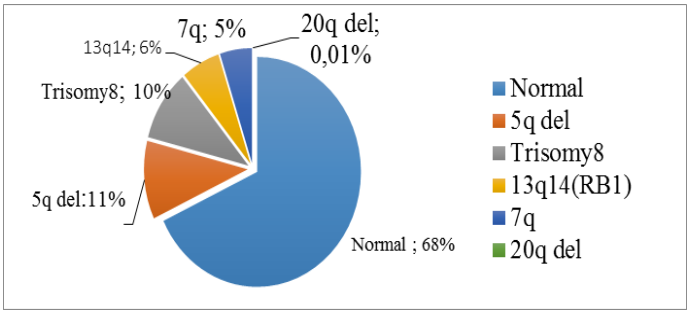
Karyotype examination was performed on 79 patients and karyotype abnormality was detected in 21 (26%) of them (Table 2).

Table 2: Cytogenetic Karyotype Results

Karyotype	Frequency	%	Cumulative %
	79	79,0	79,0
45,X,-Y[13]/46,XY[10]	1	1,0	80,0
45,X[14]/46,XY[6]	2	2,0	82,0
45,X[7]/46,XY[4]	1	1,0	83,0
45,XY,-7[9]/46,XY[14]	1	1,0	84,0
45XY-15 46 XY	1	1,0	85,0
46,XX, del(5q)(q13q31)[3]/46,XX[7]	2	2,0	87,0
46,XX[45]/45,X[4]/47,XXX[1]	2	2,0	89,0
46,XY, i(17 q) (8)/47,XY ,+8.İ(17 q) (4)	1	1,0	90,0
46,XY, del(20)(q11)	1	1,0	91,0
46,XY, del(7q)(q22)[3]/46,XY[27]	1	1,0	92,0
46,XY[15]/47,XY,+8[5]	1	1,0	93,0
46,XY[7]/46,XYdel(5)(q13p31)[2]	1	1,0	94,0
46,XY[9]/46, XY,t(3;12)(q21;q24.1)[6]	1	1,0	95,0
47,XX,+8	1	1,0	96,0
47,XY,+8	1	1,0	97,0
47,XY,+mar[15]/46,XY[5]	1	1,0	98,0
47,XYY[12]/46,XY[18]	1	1,0	99,0
(3x monosomy 21, 4x monosomy 22, 4x loss of Y chromosome)	1	1,0	100,0
Total	100	100,0	

FISH MDS panel results

The FISH MDS panel was studied from the bone marrow and contained 5 q deletions, -7 / 7q, Trisomy 8, 11q23 (MLL), 13q14 (Retinablastom1-RB1) and 20q deletion. Abnormalities were found in 16 of 77 patients (20.7%). According to the decreasing frequency ratio, 11% of the patients had 5 q deletions, 10% trisomy 8, 6% 13q14, 5% 7q, 0.01% q, and no patient had 11q23 mutations (Graph 1).



Graph 1: FISH MDS Panel Results

Karyotype and FISH analyzes revealed FISH abnormalities in 9 of 18 patients with classical karyotype abnormality and FISH was normal (50%) in 9 patients. FISH examinations of 51 patients with normal karyotype revealed abnormalities in 6 (11.8%) cases (Table 3).

Table 3: Comparison of FISH and Karyotype Results

	Cytogenetic normal	Cytogenetic abnormal	Total
Karyotype normal %	45 %88.2	6 %11,8	51 %100
Karyotype abnormal %	9 %50	9 %50	18 %100
Total	54	15	69

Polymorphism results

In the results, a rare minor allele was accepted as mutant. TET2 rs763480 polymorphism was found in 72 (72%) A/A wild type, in 24 (24%) A / T heterozygous and in 4 (4%) T / T homozygous mutant. ASXL1 rs2208131 polymorphism was found in 45 patients (45%) A/A wild type, 41 patients (41%) A/G heterozygous, and 14 patients (14%) G/G homozygous mutants. IDH1 rs11554137 polymorphism, C / C wild type in 92 patients (92%), C/T heterozygote in 8 patients (8%), but no homozygous mutant IDH1 polymorphism was detected. IDH2 rs121913503 polymorphism was found in 78 patients (78%) G/G wild type, 8 patients (8%) G/A heterozygous, 14 patients (14%) A/A homozygous mutants; IDH2 rs267606870 polymorphism was detected 100% of the C/C wild type for all 100 patients (Table 4). For this reason, only rs121913503 polymorphism was evaluated for IDH2 in subsequent analyzes.

Table 4: Polimorphism Ratios and the Allele Frequency

Mutation	Wild type	Heterozigous	Homozigous mutant	Allel frequency	
TET2 rs763480	A/A %72	A/T %24	T/T %4	A:%84	T:%16
ASXL1 rs2208131	A/A %45	A/G %41	G/G %14	A:%65	G:%35
IDH1 rs11554137	C/C %92	C/T %8	-	C:%94	T:%4
IDH2 rs121913503	G/G %78	G/A %8	A/A %14	G:%82	A:%18
IDH2 rs267606870	C/C %100	-	-	C:%100	

Evaluation of Results by Hardy-Weinberg Equlibrium

When allele frequencies were evaluated, the TET2 gene A allele was detected as 84% and T allele as 16%; Other results were as following: for ASXL1 A allele was 65% and G allele 35%, for IDH1 C allele was 96% and T allele 4%, and finally, for IDH2 G allele was 82% and A allele 18%. Compatibility between genotypes with alleles was calculated according to Hardy-Weinberg equilibrium. With the exception of the IDH2 group ($p < 0.01$), the population was found to be in equilibrium ($p > 0.05$).

Comparison of Results with Hapmap Europe Data

Since there was no control group in our study, the data were compared with HapMap European data (118 healthy individuals). TET2, ASXL1 minor allele homozygous and heterozygosity rates and allele frequencies are highly similar to

European data. While the frequency of both alleles for IDH1 is similar to the European data, there is no HapMap data for IDH2 (Table 5).

Table 5: Comparison of Our Results with HapMap European Data

Gene	Allel	Our Study	HapMap
TET2	AA AT TT A allel T allel	%72 %24 %4 %84 %16	%75 %20 %5 %85 %15
ASXL1	AA AG GG A allel G allel	%45 %41 %14 %65 %35	%41 %54 %6 %67 %33
IDH1	CC TT C allel T allel	%92 %8 %96 %4	 %95 %5
IDH2	GG GA AA G allel A allel	%78 %8 %14 %82 %18	No data

Evaluation of the relationship between SNP, demographic data and clinic

In our study, gender, diagnosis, age, WBC, Hb and PLT values were compared with SNPs. For TET2, IDH1 and IDH2 polymorphisms, there was no statistically significant difference between the groups in terms of age, WBC, Hb and PLT gender, median diagnosis (Tables 6, 7, 8), whereas there was a significant difference between the groups only in terms of age, when compared with wild type and heterozygous + homozygote ($p = 0.048$) for ASXL1 polymorphism. Heterozygous + homozygous group was found to be older than wild type (Table 9).

When comparing the polymorphism status of the genes with the data, p1 refers to the comparison of wild type, homozygous and heterozygous ones, p2 to the comparison of wild type with the total number of heterozygote and homozygote, and *; statistics could not be performed because the number of patients in the subgroups was <5.

Table 6: The Relationship of TET2 with Median Demographic and Clinical Data

TET2	Wild	Heterozygous	Homozygous	Heterozygous + Homozygous	p1 p2
Gender (M/F)	30/42	13/11	2/2	15/13	* ,37
Age (range)	65 (24-89)	65,5 (44-83)	66,5 (35-84)	65,5 (35-84)	,95 ,79
WBC 10^9/L (range)	4,51 (0,8-18,1)	6,375 (2,32-9,25)	4,055 (1,71-6,41)	6,205 (1,71-9,25)	,16 ,17
Hb gr/dl (range)	9,72 (4-13,6)	11 (7,1-14,7)	8,68 (7,7-10,7)	10,55 (7,1-14,7)	,09 ,16
PLT 10^9/L (range)	139 (12-769)	185,5 (27-381)	165,5 (35-403)	185,5 (27-403)	,98 ,96

Table 7: The Relationship of *IDH1* with Median Demographic and Clinical Data

IDH1	Wild	Heterozygous	Homozygous	P
Gender (M/F)	40/52	5/3	-	,46
Age (range)	65,5 (24-89)	61,5 (35-83)		,60
WBC 10^9/L (range)	4,63 (0,809-18,1)	5,145 (1,71-8,5)	-	,90
Hb gr/dl (range)	9,91 (4-14,7)	8,85 (4,5-11,3)		,15
PLT 10^9/L (range)	142 (12-769)	166,5 (35-302)		,70

Table 8: The Relationship of *IDH2* with Median Demographic and Clinical Data

IDH2	Wild	Heterozygous	Homozygous	Heterozygous + Homozygous	p1 p2
Gender (M/F)	38/40	2/6	5/9	7/15	* ,22
Age (range)	65,5 (24-89)	60,5 (35-76)	65 (31-83)	64,50 (31,83)	,73 ,68
WBC 10^9/L (range)	4,63 (0,809-18,1)	4,04 (1,71-15,8)	5,11 (1,85-8,78)	4,605 (0,809-15,8)	,83 ,80
Hb gr/dl (range)	9,95 (4-14,7)	8,84 (4,5-12,7)	9,76 (7,5-12,6)	9,7 (4,5-12,7)	,54 ,45
PLT 10^9/L (range)	139 (18-769)	115,5 (12-567)	210 (43-582)	184 (12-582)	,52 ,75

Table 9: The Relationship of *ASXL1* with Demographic and Clinical Data

ASXL1	Wild	Heterozygous	Homozygous	Heterozygous + Homozygous	p1 p2
Gender (M/F)	20/25	20/21	5/9	25/30	,69 1
Age (range)	61 (24-84)	66 (35-84)	68 (41-89)	67 (35-89)	0,14 0,048
WBC 10^9/L (range)	4,58 (0,809-18,1)	4,85 (2-8,37)	4,7 (1,16-9,48)	4,7 (1,16-9,48)	,98 ,90
Hb gr/dl (range)	9,87 (4-13,6)	10,12 (5,8-13,5)	9,99 (5-14,7)	9,99 (5-14,7)	,70 ,41
PLT 10^9/L (range)	165 (18-769)	156 (12-567)	137 (42-518)	139 (12-567)	,69 ,39

Comparison of SNP, karyotype and FISH results

There were no statistically significant correlations between SNP results and karyotype or cytogenetic abnormality. Only TET2 homozygous and heterozygous polymorphic group had less karyotype abnormality than wild type (p = 0.059) (Table 10).

Evaluation of prognostic effect of the results

In a 12-months follow-up, 12 of 100 patients died and 7 had AML progression. There was no significant relationship between gene polymorphisms and AML progression, median follow-up time and mortality. There was no correlation between overall survival and progression-free survival and any polymorphism. In Kaplan Meier analysis, the relationship between all gene polymorphisms and mean overall survival was not statistically significant (Tables 11, 12,13, 14).

Table 10: Comparison of SNP, Karyotype and FISH

		Wild	Heterozygous + Homozygous	P
TET2	Cytogenetic abnormal/Total Karyotype abnormal /Total	13/57 19/60	3/20 2/19	,54 0,059
ASXL	Cytogenetic abnormal/Total Karyotype abnormal /Total	9/36 11/37	7/41 10/42	,41 ,61
IDH1	Cytogenetic abnormal/Total Karyotype abnormal /Total	14/69 21/73	2/8 0/6	,66 ,18
IDH2	Cytogenetic abnormal/Total Karyotype abnormal /Total	13/59 19/63	3/18 2/16	,74 ,21

Table 11: *TET2* Prognostic Effect

TET2	Wild	Heterozygous	Homozygous	Heterozygous + Homozygous	p1 p2
Progression to AML	5/72	2/22	-	2/28	0,83 1
Median follow up (mo) (range)	25,5 (1-123)	29,5 (2-111)	46 (17-63)	31 (2-111)	,40 ,37
Mortality	11/61	0/24	1/3	1/27	,09 ,17

Table 12: *ASXL1* Prognostic Effect

ASXL1	Wild	Heterozygous	Homozygous	Heterozygous + Homozygous	p1 p2
Progression to AML	3/45	4/41	0/14	4/55	,46 ,1
Median follow up (mo) (range)	20 (1-121)	29 (2-101)	23 (11-123)	31 (2-123)	,07 ,12
Mortality	6/39	6/35	0/14	6/49	,32 ,76

Table 13: *IDH*1 Progostic Effect

IDH1	Wild	Heterozygous	Homozygous	P değeri
Progression to AML	6/92	1/8	-	0,453
Median follow up (mo) (range)	28,5 (1-123)	23,5 (12-75)		0,497
Mortality	12/80	0/8	-	0,591

Table 14: IDH2 Prognostic Effect

IDH2	Wild	Heterozygous	Homozygous	Heterozygous + Homozygous	p1 p2
Progression to AML	7/78	0/8	0/14	0/22	,34 ,34
Median follow up (mo) (range)	30 (1-123)	26 (2-75)	16,5 (2-73)	21 (2-75)	,39 ,25
Mortality	10/68	1/7	1/13	2/20	,83

The effect of results on AZA treatment response

Patients who had decreased blast count and improved cytopenias at the end of 4-6 cycles of AZA treatment were enrolled as blast responsive.

There was a higher treatment response in the heterozygous group of 20 patients who were wild type and heterozygous for the TET2 gene and who received AZA treatment (p = 0.042) (Table 15). There were no statistically significant differences in response to AZA treatment among ASXL1, IDH1 and IDH 2 polymorphisms (p = 0.64, 0.24, 0.30, respectively).

Table 15: Relationship between TET2 and Azacytidine Response

TET2 rs763480	AZA response		Total
	YES	NO	
A/A (wild type)	8	6	14
A/T(heterozygous)	0	6	6
Total	8	12	20

DISCUSSION

The median age of diagnosis of MDS was 76 years and there was a slight male majority in the literature [1]. In our study, the median age of diagnosis was 65, which is lower than literature, and there was mild female majority (1.2/1) with 55 female versus 45 male patients. Consistent with the literature, 5q syndrome patients were older and were all female [9]. Ferritin was significantly higher in MDS (p=0.012) and higher ferritin (>500) was found associated with mortality (p=0.029). However, there was no effect on overall survival (long rank p=0.059). The karyotype abnormality rate (26%) was found to be significantly lower than the 50% described in the literature. However, in our study, only 79 of the patients were examined for karyotype, which might be the reason of the low ratio.

In our study, in IDH1 rs11554137 polymorphism we found 8% heterozygous and homozygous mutant genotype was not detected. We found 14% heterozygous and 8% homozygous mutants in the IDH2 rs121913503 polymorphism. What is more, IDH2 rs267606870 heterozygous and homozygous mutant were not found in any of the patients. IDH1 / IDH2 polymorphism status (wild type-heterozygous-homozygous mutant) compared the age of diagnosis, gender, hemogram parameters (WBC-Hb-PLT), RLD, bone marrow cellularity, median blast count, denovo / secondary state, karyotype- FISH abnormality, significant relationship with WHO - IPSS group and prognostic effect (overall survival, conversion to leukemia, mortality) were not detected as well.

In a study by Chotirat et al., IDH1 rs11554137 polymorphism was detected as 9.09%, IDH2 G145G mutation was 0% and IDH2 R140Q mutation was 4.54% in MDS [10]. The IDH1 rs11554137 polymorphism ratio was similar in comparison to our study, whereas the IDH2 rs267606870 polymorphism was lower than the R140Q mutation (never detected).

Thol et al. (2010) found the IDH1 R132 mutation in MDS to be 3.6%, but no IDH2 R140 / R172 mutation was detected, whereas in our study, the IDH1 polymorphism ratio was more than the R132 mutation, and the result of R140 mutation / polymorphism for IDH2 was similar. The polymorphism of IDH2 rs121913503 was higher than R172 mutation. In the same study, the IDH1 mutation was associated with shorter survival and higher risk of AML transformation, and independent of IPSS, IDH1 was found to be a poor prognostic factor in MDS [11]. In our study, no prognostic effect was found.

Two studies have shown that IDH1 rs11554137 minor allele is prognostic in patients with AML.

Wagner et al. detected IDH1 rs11554137 minor allele was 12% in the patients with CN-AML and 11.7% in the control group and showed that the allele was a poor prognostic factor for CN-AML patients. In the same study, IDH1 R132 was 10.9% and the effect of mutation on prognosis was not detected [21].

Fasan et al. detected, IDH1 rs11554137 minor allele was 10% in AML and 9% in control group. It was found to be associated with a good prognosis in patients at the mid-risk group over 60 years of age, but that effect was not independent of other factors[12].

Jin et al. found that patients with IDH mutations responded better to demetilating agent desitabine[13]. In our study, IDH1-2 polymorphisms were not observed in patients who took decitabine.

Besides, we did not find a significant relationship between TET2 polymorphisms and prognostic factors.

When the TET2 polymorphisms were analyzed, there were two studies with rs2454206 in AML and CLL. In the AML cohort of 403 patients, Kutny et al. concluded that the TET2 rs2454206 minor allele ($TET2^{AG/GG}$) was associated with better survival than common allele ($TET2^{AA}$) [22]. Hernández et al. evaluated TET2 polymorphism with new generation sequencing in CLL and found that rs2454206 was the most common SNP. They suggested that it might be associated with the development of CLL[14]. Furthermore, in a recent study Chen et al showed that TET2 mutations were related to poor overall survival in AML [23].

Palomero et al. found the TET2 mutation ratio was 22.9% in MDS and there was no significant difference between the WHO-FAB-IPSS classes and their prevalence. Also there was no significant difference between treatment and TET2 mutation. In our study, we did not find a corelation between TET2 polymorphism and WHO-IPSS class.

Moran-Crusio et al. TET2 mutation has been shown to be a favorable prognostic factor for IPSS-independent MDS[15]. However, in a larger study of 320 patients enrolled by Smith et al., TET2 with a frequency of 12% mutation was not associated with a general prognosis[16]. Also in our study, we did not detect the prognostic effect of TET2 polymorphism.

Itzykson et al. showed that patients with TET2 mutation respond better to AZA treatment than the wild type [5]. In our study, 20 patients receiving AZA therapy were found to have higher TET2 heterozygous polymorphism than the wild type ($p = 0.042$). In terms of TET2 polymorphism, there was no difference between the clinical and demographic characteristics of the wild and heterozygous group, but less karyotype abnormality was observed in the TET2 polymorphic group ($p = 0.059$). Loss of TET2 function may make cells sensitive to hypometylating drugs. The change in the TET2 methylation state is associated with response to transient drugs and can be used as a potential therapeutic marker [17].

In our study, we did not find a relationship between ASXL1 polymorphism status (wild type/ heterozygote/ homozygous mutant) and clinical or demographic data. We found only the mean age of ASXL1 heterozygote + homozygous group significantly higher ($p = 0.048$) than wild type group. Uslu et al.[18] and Brecqueville et al. [20] found the ASXL1 mutation in MPN to be associated with advanced age.

Thol et al. showed that ASXL1 mutation correlated with aggressive disease and poor survival. In this study, ASXL1 mutation was found to be associated with shortening progression time to AML in MDS and it was accepted as an independent prognostic factor [19]. In our study, the ASXL1 polymorphism was not associated with prognosis.

Although the pathogenesis of MDS has not been fully elucidated, the role of epigenetic changes in pathogenesis has been demonstrated. Polymorphism studies can be carried out after determining that the polymorphisms of IDH1, IDH2, TET2 and ASXL1 are prognostic factors in AML. After further studies, the prognostic significance of these genes can be used in scoring systems, treatment selection, and prediction of treatment response.

Conflict of interest

The authors declare that there is no conflict of interest.

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