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EVALUATION OF MIR-145 AND MIR-146A AS POTENTIAL BIOMARKERS FOR DIAGNOSIS OF MYELODYSPLASTIC SYNDROME

MİYELODİSPLASTİK SENDROM TANISINDA MIR-145 VE MIR-146A'NIN POTANSİYEL BİYOBELİRTEÇ OLARAK DEĞERLENDİRİLMESİ

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ABSTRACT

Objective: Myelodysplastic syndromes (MDS) are a group of heterogeneous hematopoietic stem cell disorders characterized by ineffective hematopoiesis, bone marrow dysplasia, and peripheral cytopenias. microRNAs (miRNAs) are small non-coding RNAs that play key roles in post-transcriptional regulation of gene expression and have been determined potential in disease diagnostics and therapeutics owing to their stability. Recent evidence suggests that haploinsufficiency of the miR-145 and miR-146a, encoded from 5q Common Deleted Region (CDR) may contribute to the phenotype in MDS. Although, interstitial del(5q) is the most common chromosomal abnormality in MDS, these findings are inconsistent in Turkish patients. Therefore, we aimed to investigate assess the diagnostic value of miR-145/miR-146a and their relation with del(5q) or monosomy 5 in MDS.

Methods: In order to determine the association between del(5q) and expression miR-145/miR-146a, conventional cytogenetics (CC), FISH, and qRT-PCR methods were performed for 24 patients with MDS and 20 healthy individuals. Additionally, ROC curves were generated to evaluate putative diagnostic value of miRNAs.

Results: Cytogenetic examination revealed clonal cytogenetic abnormalities in 43.4% of cases. miR-146a decreased in 23 of 24 patients regardless of chromosome 5 abnormalities ($p<0.001$), expression level of miR-145 was statistically nonsignificant. miR-146a levels performed well as a diagnostic biomarker, discriminating MDS patients from controls with an area under the ROC curve (AUC) of 0.942, 83.3% sensitivity.

Conclusion: miR-146a may be used as a biomarker in diagnosis of MDS and may help to identify new treatment targets. In addition, we suggest that CC and FISH methods should be performed together in MDS.

Keywords: Myelodysplastic syndrome; diagnostic biomarkers; miR-146a; ROC curve

ÖZ

Amaç: Miyelodisplastik sendrom (MDS), verimsiz hematopoez, kemik iliği displazisi ve periferik sitopeni ile karakterize edilen heterojen bir grup hematopoetik kök hücre bozukluğudur. mikroRNA'lar (miRNA'lar), gen ifadesinin post-transkripsiyonel düzenlenmesinde kilit rol oynayan küçük kodlamayan RNA'lardır ve stabiliteleri sayesinde hastalık tanısında ve tedavisinde potansiyelleri belirlenmiştir. Son çalışmalar, 5q Yaygın Delesyon Bölgesi'nden (YDB) kodlanan miR-145 ve miR-146a'nın haplo-yetmezliğinin MDS'deki fenotipe katkıda bulunabileceğini düşündürmektedir. MDS'de en sık gözlenen kromozom anomalisi interstisyel del(5q) olmasına rağmen, bu bulgular Türkiye popülasyonundaki örnekler ile tutarsızdır. Bu nedenle, MDS'de miR-145/miR-146a'nın tanısal kullanım değerini ve bu miRNA'ların del(5q) ve/veya monozomi 5 ile ilişkisini değerlendirmeyi amaçladık.

Yöntem: del(5q) ile miR-145/miR-146a ekspresyonu arasındaki ilişkiyi belirlemek için MDS'li 24 hasta ve 20 sağlıklı kontrol için konvansiyonel sitogenetik (CC), FISH ve qRT-PCR yöntemleri uygulanmıştır. Ek olarak, miRNA'ların tanısal değerini değerlendirmek için ROC eğrileri oluşturulmuştur.

Bulgular: Sitogenetik incelemeler, vakaların %43,4'ünde klonal sitogenetik anomali olduğunu gösterdi. miR-146a ifadesi, 5. kromozom anomalilerinden bağımsız olarak 24 hastanın 23'ünde azalmaktaydı ($p<0,001$), miR-145'in ekspresyon düzeyi istatistiksel olarak anlamlı değildi. miR-146a ifade seviyesi, MDS hastalarını kontrollerden ayırmada 0,942 ROC eğrisi altında kalan alan (AUC) değeri ve %83,3 hassasiyet değeri ile iyi bir tanısal biyobelirteç performansı sergiledi.

Sonuç: miR-146a, MDS tanısında bir biyobelirteç olarak kullanılabilir ve yeni tedavi hedeflerinin belirlenmesine yardımcı olabilir. Ayrıca MDS'de CC ve FISH yöntemlerinin birlikte yapılmasını öneriyoruz.

Anahtar Kelimeler: Miyelodisplastik sendrom; tanısal biyobelirteç; miR-146a; ROC eğrisi

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Introduction

Myelodysplastic syndromes (MDS) are clonal heterogeneous hematopoietic stem cell (HSC) malignancies characterized by dysplastic cells in bone marrow and cytopenia in peripheral blood due to inefficient hematopoiesis.[1,2] One third of the cases progress to acute myeloid leukemia (AML).[3] MDS may occur *de novo* or secondary as a consequence of chemotherapy/radiotherapy in cancer patients.[4] Although MDS are rare in childhood, can be associated with some genetic diseases such as Down syndrome, Fanconi anemia, and neurofibromatosis. Increased incidence of the MDS with aging suggests that genetics and environmental factors cause cumulative damage in bone marrow cells.[5,6]

The exact mechanisms leading to ineffective hematopoiesis in MDS remain elusive yet. However, it is known that chromosomal defects and genetic alterations play a main role in the etiopathogenesis of MDS.[7,8] Clonal chromosomal abnormalities are seen in 30-50% of MDS patients, among them del(5q), −7, and +8 are frequent which have been included in the more vigorous prognostic scoring systems of the disease.[9]

In particular, isolated 5q deletion was reported in many studies and approximately 1.5-megabase Common Deleted Region (CDR) has been mapped to 5q33-q35 location, and predicted to house several hematopoiesis related genes.[10] In addition, many gene variations and microRNAs (miRNAs) encoded on this region with altered gene expression have been associated with MDS. Especially, previous reports have indicated that miR-145 and miR-146a are possible cause of haploinsufficiency.[11,12] Moreover, knockdown studies in mouse have shown that loss of both miR-145 and miR-146a resulted in increased platelet counts associated with dysplastic megakaryopoiesis and neutropenia.[12] Taken all together, our aim was to evaluate assess the diagnostic value of miR-145/miR-146a and their relation with 5q deletion or monosomy 5 in MDS.

Materials and Methods

Patients Recruitment

In the present study, leukocyte cells were obtained from the bone marrow aspiration materials of 24 newly diagnosed and untreated MDS patients (mean age: 65.37±9.50 years, 17 female and 7 male) and peripheral blood samples of 20 healthy subjects (mean age: 43.40±7.37 years, 12 female and 8 male).

Conventional Cytogenetic (CC) Analysis

Bone marrow aspiration materials were cultured for 24h and 48h, and peripheral blood samples were cultured for 72h in RPMI-1640 medium. Peripheral blood samples were stimulated with Phytohemagglutinin. As a conventional method, colchicine (0.1 µg/ml final concentration) was used to stop division during metaphase stage. Cytogenetic analyses were performed on specimens using a Trypsin-Leishman (GTL) banding and at least 20 metaphases were analyzed, when available. The chromosome identification and karyotype description were determined according to the International System for Human Cytogenomic Nomenclature (ISCN) 2016 criteria.[13]

Fluorescence in Situ Hybridization (FISH)

FISH on interphase nuclei was performed according to the manufacturer's instructions using Vysis LSI CSF1R SpectrumOrange/D5S23, D5S721 SpectrumGreen probes (Abbott Molecular, Abbott Park, IL, USA) to detect 5q33-34 region deletions. The slides were counterstained with DAPI. When possible, at least 200 interphase nuclei were analyzed. Cell images were captured under 100X immersion magnification with ISIS (Metasystems, Isis Fluorescence Imaging) program using Olympus BX51 microscope for FISH analysis.

Gene Expression Analysis

Total RNA was extracted using Direct-zol™ RNA MiniPrep (Zymo Research, CA, USA) according to the manufacturer's recommendations. The quality and quantity of RNA was evaluated using the Multiskan GO (Thermo Fisher Scientific, Boston, MA, USA).

miRNA analysis was conducted using 200 ng of total RNA as a template for reverse transcription with miRNA-specific RT-oligonucleotide. miRNA-spesific cDNA conversions and qRT-PCRs were performed using EPIK™ miRNA Select Hi/Lo-ROX Kit (Bioline, London, UK) according to the manufacturer's protocols for miR-145, miR-146a and U6 snRNA as a reference gene. cDNA conversion was performed at 42 °C for 30 min, followed by heat-inactivation of the reverse transcriptase at 90 °C for 5 min. PCR amplification conditions for miRNAs were as follows: One cycle of initial denaturation step at 95°C for 10 min, and 40°C for 5 min, followed by 40 cycles of amplification step at 95°C for 10 s, 60°C for 30 s. Gene expression levels were performed using the Bio-Rad CFX96 instrument. Relative gene expression rates were calculated using the $2^{-\Delta\Delta CT}$ method.[14]

Statistical Analysis

In the present study, whether the data were distributed normally was tested with Shapiro-Wilk test. In comparing the data with normal distribution between two independent groups, the independent sample t-test was used and the non-normal distribution of the data between two independent groups was evaluated with the Mann-Whitney U test. Statistical analyses were performed at GraphPad Prism 8.0 program (GraphPad Software, Inc., CA, USA) with a significance level of 0.05 and 95% confidence level.

Receiver Operating Characteristic (ROC) curves were generated to establish Cut-Off values using MedCalc Version 19.6.4 program. Cut-off values were determined by Youden J Index, and sensitivity, specificity, positive predictive value, negative predictive value was calculated.[15-17]

For FISH analyses, *BetaInv* method was used and cut-off values were established by *BetaInv* function.[18] Briefly, *BetaInv* is a method that calculates the 95% of upper confidence limit of binomial distribution. Mean and standard deviation methods use Gaussian distribution. However, FISH results are not suitable for this, therefore, binomial curve must be used. In *BetaInv* method, confidence of interval is selected as 95%, $p<0.05$ is significant.

BetaInv ("Confidence Interval";" False positive cell number +1"; "Examined cell number").

Results

Cytogenetic Analyses

Karyotypes of the cases were presented in Table 1.

Table 1. CC and FISH analysis results in patients. Cut-off value was set as 8% for del(5q) and 5% for monosomy 5

Patient	Age	Sex	CC	FISH (%)	
				del(5q)	-5
1	61	M	37~45,XY,-Y[9],-18[3][cp11]/46,XY[6]	2.9	1.5
2	63	F	46,XX[1], nca[a][2]	np[b]	
3	72	M	46,XY[1], nca[3]	9.1	1.0
4	69	M	46,XY[2], nca[3]	3.7	1.9
5	52	M	39~45,X,-Y[4],-6[3],-15[3],-22[4][cp7]/46,XY[11]	3.9	3.4
6	64	F	46, XX[12], nca[8]	5.4	4.4
7	47	F	40~51,XX,-8[3],-14[3],-15[5],-17[4],-20[3][cp8]/46,XX[16]	2.8	2.3
8	78	M	36~45,X,-Y[5],-5[3],-9[3],-10[3],-11[3],-18[3],-20[3], -22[3][cp11]/46,XY[10]	2.0	3.0
9	82	F	35~45,XX,-5[3],-19[3],-20[4],-21[3][cp6]/46,XX[6]	1.9	1.0
10	61	F	27~33,X,+X[2],+1[2],+3[3],+4[2], +7[2],+9[2],+10[3],+13[3], +14[2],+15[2],+16[4],+19[2],+21[2][cp4]/42~45,XX,-9[3], -12[3],-18[3],-21[4][cp8]/46,XX[8]	3.1	0.9
11	66	F	nca[3]	np	
12	55	F	-[c]	np	
13	84	M	27~34,X,+2[2],+6[2],+13[2],+18[2],+21[2],+22[2][cp2]/ 39~45,XY,-16[4],-21[3][cp7]/46,XY[16]	3.4	3.8
14	55	M	28~34,X,+Y[2],+3[2],+5[2],+8[2], +12[2][cp2]/ 36~45,XY,-17[3],-21[3][cp8]/46,XY[11]	5.6	7.5
15	50	F	-	2.9	6.7
16	79	F	46,XX[2]	1.4	5.8
17	64	F	-	2.0	2.5
18	65	F	-	np	
19	60	F	37~46,XX,-7[8],del(21)(q22)[3],+mar1[6][cp10]	7.5	0.5
20	75	F	46,XX[1]	4.5	1.4
21	69	F	46,XX,ins(X;?)(p11.2;??)[3]/35~45,XX,-17[4],-19[3], -22[3][cp7]/46,XX[10]	1.0	4.4
22	69	F	46,XX[20], nca[7]	3.4	1.9
23	66	F	46,XX[10], nca[8]	np	
24	63	F	-	np	

CC: Conventional Cytogenetics, FISH: Fluorescence in situ hybridization. [a] non-clonal abnormalities, [b]not performed, [c]no quality metaphase to evaluate

Despite monosomy 5 was detected in two patients (#8 and #9), del(5q) was not present in any patients. Monosomy 21 was detected in four patients, also monosomy 17, 18, 20, 22 and Y were seen in three patients each. However, numerical chromosomal changes with gain or loss were detected in almost all chromosomes. The structural abnormalities were del (21)(q22), ins (X;?)(p11.2;??) and +mar as well. Normal and abnormal i.e., numerical, or structural changes were compiled in Figure 1.

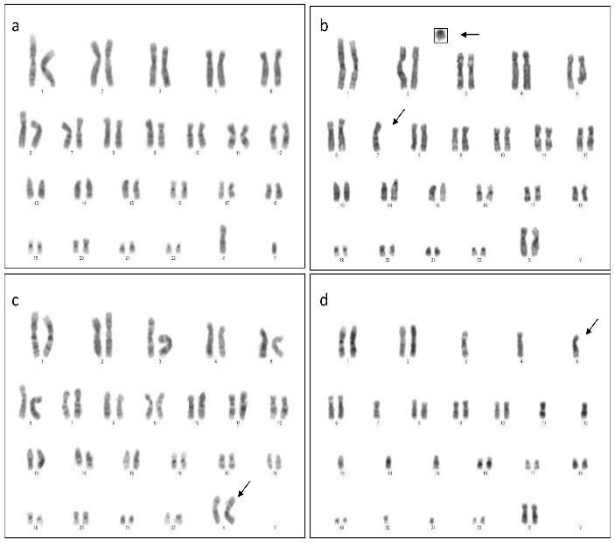

Figure 1. G-banding karyotype results; a. 46,XY (Control #11). b. 46,XX,-7,+mar1 (Patient #19). c. 46,XX,ins(X;?)(p11.2;??) (Patient #21). d. 35,XX,-3,-4,-5,-7,-11,-12,-13,-14,-15,-20,-21 (Patient #9)

FISH Analyses

To confirm and evaluate the significance of the results, FISH was performed in 20 healthy subjects and the data were used in *Betainv* function to calculate the Cut-off values. Cut-off value was set as 8% for del(5q) and 5% for monosomy 5. FISH was successfully carried out in 18 patients. However, due to inadequate material, FISH was not performed in six patients. Consequently, we have detected a significant deletion signal for del(5q) in one patient (#3) and for monosomy 5 in three patients (#14, #15, and #16). The deletion signals were within normal range for other patients (Table 1). Normal, del(5q), and monosomy 5 signals were detected by FISH as were shown in Figure 2.

Gene Expression Analyses

To investigate gene expression levels of miR-145 and miR-146a, qRT-PCR was conducted. miR-146a expression significantly decreased in 23 of 24 patients' bone marrow samples ($p<0.0001$) (decreased by fold change between 0.01-0.80, increased by 1.42-fold in one patient), while expression of miR-145 increased in 15 (62.5%) patients, decreased in six (25%), and did not change in three (12.5%) patients' bone marrow samples compared to healthy control's peripheral blood samples. ROC curve analysis showed an AUC of 0.942 for miR-146a detailed

in Table 2, there was no statistically significant value for miR-145 (p>0.05), compiled in Figure 3.

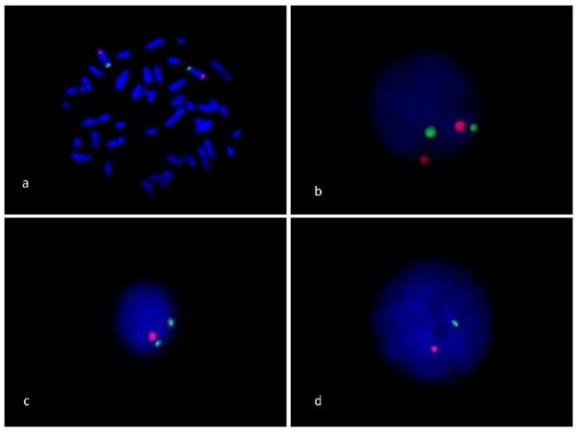

Figure 2. FISH images; SpectrumGreen probe on 5p15.2 region (G) and SpectrumOrange probe on 5q33-34 region (as mentioned red (R)). a. Normal metaphase cell (2G2R). b. Normal interphase cell (2G2R). c. Monoallelic deletion of chromosome 5q (2G1R). d. Monosomy of chromosome 5 (1G1R).

Table 2. Diagnostic biomarker features of miR-145 and miR-146a in MDS.

	SE (%)	SP (%)	PPV (%)	NPV (%)	Accuracy	AUC	p
miR-145	66.67	65.0	69.6	61.9	0.650	0.623	0.163
miR-146a	83.33	100.0	100.0	84.0	0.909	0.942	<0.001

SE: sensitivity, SP: specificity, PPV: positive predictive value, NPV: negative predictive value, AUC: area under the ROC curve

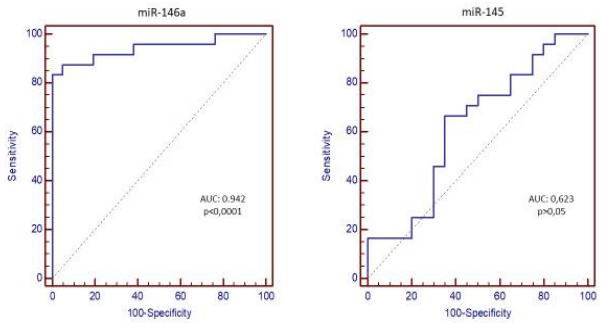

Figure 3. Diagnostic performance analysis by ROC curves (Receiver Operating Characteristic) examining AUC (area under the ROC curve) for miR-145 and miR-146a.

Discussion

MDS are clinically and genetically heterogeneous group of clonal hematological diseases developing due to defective progenitor bone marrow cells. Additionally, recent studies indicated that dysfunctions of hematopoietic stem cells, dysregulation of inflammatory and innate immunity, and complex genomic aberrations are related to MDS. [19]

Myelodysplasia occurs when somatic mutations originate from one or more hematopoietic lineages and suppress the cell differentiation.[20,21] Development of the abnormal stem cell clone, which is genetically unstable because of mutations, cause ineffective hematopoiesis with increased apoptosis. This condition is presented by cytopenia in the affected hematopoietic lineages, leading to leukemia in one third of cases.[21,22]

According to literature, clonal chromosomal abnormalities have been detected in 30-50% of patients with MDS. In particular, -5/del (5q), -7/del (7q), +8 and -Y findings were common in cytogenetic studies in large cohorts involving MDS cases.[9] Isolated del(5q) is associated with a good prognosis, long overall survival, and a low risk of leukemic evolution.[23] Herein, clonal cytogenetic abnormality was detected in 43.4% of cases; among them 16.6% had -21, and 12.5% had -17, -18, -20, -22, -Y, respectively. Numerical changes were observed in almost all chromosomes, and -5 was present in two cases (8.3%). However, clonal structural changes were rarely detected, i.e. del(21)(q22) and marker chromosome in one case, ins(X;?)(p11.2;??) in one case, and recurrent chromosomal gaps and breaks in another case were detected.

While our data are consistent with the results of previous reports showing clonal numerical aberrations, mentioned common chromosomal abnormalities were inconsistent. However, we also compared our results with regional studies conducted in Turkey as well. While Yilmaz et al. could not detect -5/del(5q) using CC, they were able to show del(5q) in 3 of 26 MDS patients by FISH.[24] In our previous study, we performed a retrospective CC study including 221 untreated MDS patients, and we detected cytogenetic abnormalities in 44.8% patients, which were; -Y (7.7%), -18 (7.7%), -21 (6.8%), -7 (6.3%), -22 (5.4%), -5 (4.5%), +8 (4.5%), -19 (4.5%), del(5q) (1.3%), del (20q) (0.9%), respectively.[25] Our results are coherent with the results of the studies conducted in similar geographical location and population.[24,25] These results support the study of Kawankar et al., suggesting that the frequency of chromosomal aberration may vary according to geographical and ethnic differences.[9]

As an additional output of our study, we recommend that FISH and CC should be combined in MDS and other genetically heterogeneous diseases. Even though, CC is still the gold standard in MDS as it provides a complete picture of the chromosomes, it may be difficult to perform CC in some cells with poor chromosome morphology, and in those with low *in vitro* mitotic activity such as cancer cells. In addition, cytogenetic analysis requires at least 20 metaphases for a reliable result. However, in FISH studies, metaphase is not must and interphase can be used, instead.[26] On the other hand, using FISH alone allows detection of certain abnormalities and some genetic changes may be overlooked. To overcome this problem combining these methods is useful tool. Furthermore, today, additional techniques such as flow-cytometry and genomic sequencing are helping for the precision diagnosis of MDS.[19]

In our study, we did not observe del(5q) in any patients, but detected -5 in two cases (#8 and #9) by CC. However, FISH analysis showed that the number of cells with -5

were lower than the cut-off value in both cases. On the other hand, by FISH, we observed del(5q) in one (#3) and -5 in three (#14, #15, and #16) patients and only one of these cases (#14) had enough metaphases for CC analysis, while the others had either none or very few metaphases.

In the literature, one of the most common cytogenetic alterations is isolated 5q deletion in MDS. Interstitial deletion of chromosome 5q is defined as related to the phenotype of MDS such as refractory anemia, variable neutropenia. The CDR on chromosome 5q has been mapped to band q33.1-q35, approximately 1.5 megabases.[12] This CDR region contains 40 coding genes including tumor suppressor gene *SPARC* and ribosomal subunit gene *RPS14* that are considered to play roles in the pathogenesis of myeloid malignancies.[27]

Also, in this region (5q31-35), 13 miRNA clusters have been mapped, and they have been shown to play an important role in the development of specific clinical features of the disease and malignant clone dominance.[28] Among them, miR-145 and miR-146a are seemed to be particularly important according to many studies.[27,29] Starczynowski et al. have investigated the possible roles of miRNAs in 5q- syndrome, a subtype of MDS, and they have found that the loss of miR-145 and miR-146a is significantly associated with 5q- syndrome. miR-145 and miR-146a are found abundantly in hematopoietic stem cells and they have been suggested to be associated with *TIRAP* (Toll-Interleukin-1 Receptor Domain-Containing Adaptor Protein) and *TRAF6* (Tumor Necrosis Factor Receptor-Associated Factor-6), important actors of the immune pathway.[12] Several studies have shown that loss of miR-146a with 5q deletion increases *TRAF6* mRNA levels and translation, while loss of *TIFAB* (TRAF-interacting protein with forkhead-associated domain B) increases TRAF6 protein stability, thereby overexpressing and activating *TRAF6* in HSCs.[30-32] Furthermore, germline knockout of mouse studies has indicated the loss of miR-146a resulted in an early onset of the myeloid expansion in the bone marrow, and seen progression to lymphomas, bone marrow failure, and myeloid leukemia.[30]

Since noticed that miRNAs displayed high stability in clinical samples miRNAs have been considered as potential disease biomarkers.[35] As is known, ROC curve analysis is used as the most popular graphical tool for assessing the diagnostic power of a biomarker through calculating the sensitivity and the specificity.[36] To date, many miRNAs have been determined as disease biomarker with early disease diagnosis and effective prognostic monitoring.[15-17] Therefore, we used ROC analysis to define possible biomarker in MDS, as well.

Previous studies have shown that miR-146a expression decreases in 25% of MDS patients regardless of cytogenetic condition.[33,34] In our study, the statistically significant decrease in miR-146a expression in 23 of 24 patients regardless of 5q deletion or monosomy 5. Also, alteration of miR-146a expression showed significant biomarker performance in our cohort with an AUC of 0.902, with 83.3% sensitivity. Thus, we recommend that miR-146a as a potential diagnostic biomarker for MDS patients regardless of cytogenetic findings according to our study and many literature sources.

These results must be considered in some limitations that our study cohort is relatively small, and our control group samples were peripheral leukocyte cause unfortunately we could not reach bone marrow samples from healthy individuals.

Conclusions

miR-146a may be considered as a remarkable biomarker in diagnosis of MDS. Genetic and epigenetic mechanisms that may alter expression of miR-146a and their effect on molecular signal pathway(s) should be examined in detail to identify new treatment targets. Large-scale further research in larger cohorts will be efficient in understanding the etiopathogenesis of the MDS and evaluating possible treatment options.

Statement of Ethics

This study was approved by the Ethics Committee of Istanbul University-Cerrahpasa, Cerrahpasa Faculty of Medicine (no:16.11.2016-414947). All participants gave their written informed consent before the study.

Conflict of Interest Statement

Authors declare that there is no conflict of interest regarding this work.

Author Contribution

Authors contributed equally to this work.

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References

1. Campbell LJ. *Cancer cytogenetics: methods and protocols.* Humana Press; 2011. ISBN: 9781617790744
2. Garcia-Manero G. Myelodysplastic syndromes: 2014 update on diagnosis, risk-stratification, and management. *Am J Hematol.* 2014;89(1):97-108. doi:10.1002/ajh.23642
3. Zahid MF, Malik UA, Sohail M, Hassan IN, Ali S, Shaukat MHS. Cytogenetic Abnormalities in Myelodysplastic Syndromes: An Overview. *Int J Hematol Oncol Stem Cell Res.* 2017;11(3):231-239.
4. Corey SJ, Minden MD, Barber DL, Kantarjian H, Wang JC, Schimmer AD. Myelodysplastic syndromes: the complexity of stem-cell diseases. *Nat Rev Cancer.* 2007;7(2):118-129. doi:10.1038/nrc2047
5. Charles Wiener M, Kasper DL, Fauci AS, et al. Harrison's Principles of Internal Medicine Self-Assessment and Board Review. 2012. ISBN: 9781260470093
6. Hoffman R, Benz Jr EJ, Silberstein LE, Heslop H, Anastasi J, Weitz J. *Hematology: basic principles and practice.* Elsevier Health Sciences; 2013. ISBN: 9780323357623
7. Bernasconi P. Molecular pathways in myelodysplastic syndromes and acute myeloid leukemia: relationships and

distinctions-a review. *Br J Haematol.* 2008;142(5):695-708. doi:10.1111/j.1365-2141.2008.07245.x

8. Haase D, Germing U, Schanz J, et al. New insights into the prognostic impact of the karyotype in MDS and correlation with subtypes: evidence from a core dataset of 2124 patients. *Blood.* 2007;110(13):4385-4395. doi:10.1182/blood-2007-03-082404
9. Kawankar N, Vundinti BR. Cytogenetic abnormalities in myelodysplastic syndrome: an overview. *Hematology.* 2011;16(3):131-138. doi:10.1179/102453311X12940641877966:
10. Giagounidis AA, Germing U, Aul C. Biological and prognostic significance of chromosome 5q deletions in myeloid malignancies. *Clin Cancer Res.* 2006;12(1):5-10. doi:10.1158/1078-0432.CCR-05-1437
11. Visconte V, Tiu RV, Rogers HJ. Pathogenesis of myelodysplastic syndromes: an overview of molecular and non-molecular aspects of the disease. *Blood Res.* 2014;49(4):216-227. doi:10.5045/br.2014.49.4.216
12. Starczynowski DT, Kuchenbauer F, Argiropoulos B, et al. Identification of miR-145 and miR-146a as mediators of the 5q- syndrome phenotype. *Nat Med.* 2010;16(1):49-58. doi:10.1038/nm.2054
13. McGowan-Jordan J. *ISCN 2016: An International System for Human Cytogenomic Nomenclature (2016): Recommendations of the International Standing Committee on Human Cytogenomic Nomenclature Including New Sequence-based Cytogenetic Nomenclature Developed in Collaboration with the Human Genome Variation Society (HGVS) Sequence Variant Description Working Group.* Karger; 2016. ISBN: 9783318058574
14. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods.* 2001;25(4):402-408. doi:10.1006/meth.2001.1262.
15. Wang J, Zhu X, Xiong X, et al. Identification of potential urine proteins and microRNA biomarkers for the diagnosis of pulmonary tuberculosis patients. *Emerg Microbes Infect.* 2018;7(1):63. doi:10.1038/s41426-018-0066-5
16. Martins-Ferreira R, Chaves J, Carvalho C, et al. Circulating microRNAs as potential biomarkers for genetic generalized epilepsies: a three microRNA panel. *Eur J Neurol.* 2020;27(4):660-666. doi:10.1111/ene.14129
17. Pires-Luis AS, Costa-Pinheiro P, Ferreira MJ, et al. Identification of clear cell renal cell carcinoma and oncocytoma using a three-gene promoter methylation panel. *J Transl Med.* 2017;15(1):149. doi:10.1186/s12967-017-1248-y
18. Arsham MS, Barch MJ, Lawce HJ. *The AGT cytogenetics laboratory manual.* John Wiley & Sons; 2017. ISBN: 9781119061229
19. Garcia-Manero G, Chien KS, Montalban-Bravo G. Myelodysplastic syndromes: 2021 update on diagnosis, risk stratification and management. *Am J Hematol.* 2020;95(11):1399-1420. doi:10.1002/ajh.25950
20. Loken MR, van de Loosdrecht A, Ogata K, Orfao A, Wells DA. Flow cytometry in myelodysplastic syndromes: report from a working conference. *Leuk Res.* 2008;32(1):5-17. doi:10.1016/j.leukres.2007.04.020
21. Pellagatti A, Boultwood J. The molecular pathogenesis of the myelodysplastic syndromes. *Eur J Haematol.* 2015;95(1):3-15. doi:10.1111/ejh.12515
22. Macedo LC, Silvestre AP, Rodrigues C, et al. Genetics factors associated with myelodysplastic syndromes. *Blood Cells Mol Dis.* 2015;55(1):76-81. doi:10.1016/j.bcmd.2015.04.003
23. Azevedo RS, Belli C, Bassolli L, et al. Age, Blasts, Performance Status and Lenalidomide Therapy Influence the Outcome of Myelodysplastic Syndrome With Isolated Del(5q): A Study of 58 South American Patients. *Clin Lymphoma Myeloma Leuk.* 2022;22(1):e1-e6. doi:10.1016/j.clml.2021.07.026
24. Yilmaz Z, Sahin FI, Kizilkilic E, Karakus S, Boga C, Ozdogu H. Conventional and molecular cytogenetic findings of myelodysplastic syndrome patients. *Clin Exp Med.* 2005;5(2):55-59. doi:10.1007/s10238-005-0066-3
25. Deviren A, Gursel IM, Yılmaz S, Hacıhanefioglu S. Cytogenetic Evaluation in 221 Untreated Patients with Myelodysplastic Syndrome/Tedavi Almamis 221 Miyelodisplastik Sendromlu Hastada Sitogenetik Degerlendirme. *J Türkiye Klinikleri Tip Bilimleri Dergisi.* 2012;32(1):15. doi:10.5336/medsci.2010-20908
26. Kokate P, Dalvi R, Koppaka N, Mandava S. Prognostic classification of MDS is improved by the inclusion of FISH panel testing with conventional cytogenetics. *Cancer Genet.* 2017;216-217:120-127. doi:10.1016/j.cancergen.2017.05.004
27. Venugopal S, Mascarenhas J, Steensma DP. Loss of 5q in myeloid malignancies - A gain in understanding of biological and clinical consequences. *Blood Rev.* 2021;46:100735. doi:10.1016/j.blre.2020.100735
28. Li J. Myelodysplastic syndrome hematopoietic stem cell. *Int J Cancer.* 2013;133(3):525-533. doi: 10.1002/ijc.27896
29. Chan O, Talati C, Sallman D, List A. Biology and Pathophysiology of MDS with del (5q). In: *Diagnosis and Management of Myelodysplastic Syndromes.* Springer; 2020:43-54. doi:10.1007/978-3-030-51878-3_3
30. Varney ME, Niederkorn M, Konno H, et al. Loss of Tifab, a del(5q) MDS gene, alters hematopoiesis through derepression of Toll-like receptor-TRAF6 signaling. *J Exp Med.* 2015;212(11):1967-1985. doi:10.1084/jem.20141898
31. arney ME, Choi K, Bolanos L, et al. Epistasis between TIFAB and miR-146a: neighboring genes in del(5q) myelodysplastic syndrome. *Leukemia.* 2017;31(2):491-495. doi:10.1038/leu.2016.276
32. Barreyro L, Chlon TM, Starczynowski DT. Chronic immune response dysregulation in MDS pathogenesis. *Blood.* 2018;132(15):1553-1560. doi:10.1182/blood-2018-03-784116
33. Starczynowski DT, Morin R, McPherson A, et al. Genome-wide identification of human microRNAs located in leukemia-associated genomic alterations. *Blood.* 2011;117(2):595-607. doi:10.1182/blood-2010-03-277012
34. Sokol L, Caceres G, Volinia S, et al. Identification of a risk dependent microRNA expression signature in myelodysplastic syndromes. *Br J Haematol.* 2011;153(1):24-32.doi:10.1111/j.1365-2141.2011.08581.x
35. Hydbring P, Badalian-Very G. Clinical applications of microRNAs. *F1000Res.* 2013;2:136. doi:10.12688/f1000research.2-136.v3
36. Hsu MJ, Chang YC, Hsueh HM. Biomarker selection for medical diagnosis using the partial area under the ROC curve. *BMC Res Notes.* 2014;7:25. doi:10.1186/1756-0500-7-25