Original Article

DOI: 10.14235/bas.galenos.2021.5447
Bezmialem Science 2022;10(4):453-60

mir210 and BCL2 Expressions in Patients with Acute Myeloid Leukemia

Akut Myeloid Lösemi Hastalarında mikroRNA-210 ve *BCL-2* Ekspresyon Seviyeleri

Handenur GÖKTAN[1], Rahiye Dilhan KURU[1], Şükriye YILMAZ[1], Merve ALAYLIOĞLU[1], Erdinç DURSUN[1], Duygu GEZEN AK[1], Ayşe SALİHOĞLU[2], Ayhan DEVİREN[1]

[1]İstanbul University-Cerrahpaşa Cerrahpaşa Faculty of Medicine, Department of Medical Biology, İstanbul, Turkey
[2]İstanbul University-Cerrahpaşa Cerrahpaşa Faculty of Medicine, Department of Hematology, İstanbul, Turkey

ABSTRACT

Objective: Acute myeloid leukemia (AML) is a heterogeneous, malignant disease, characterized by disruption of differentiation of hematopoietic stem cells. The effect of microRNAs (miRNA)-210 on the prognosis of AML is a subject of research. *BCL-2* is a protooncogene encoding a protein that inhibits apoptosis. Overexpression of *BCL-2* is seen in AML cells. The presence or absence of cytogenetic abnormalities is an important prognostic marker in AML. In our study, we aimed to compare the effect of miRNA-210 and *BCL-2* expressions on the prognosis of AML and the relationship with the cytogenetic findings.

Methods: Cytogenetic analyzes were performed in bone marrow and/or peripheral blood samples taken from patients with AML and healthy individuals. miR-210 and *BCL-2* mRNA levels were determined by quantitative real-time polymerase chain reaction method.

Results: No abnormality was found in healthy individuals. Clonal structural and numerical anomalies were detected in some patients with AML. *BCL-2* mRNA expression levels of leukocytes and bone marrow samples of patients with AML were higher than that of the leukocytes of healthy individuals. miR-210 levels did not differ between patients and healthy individuals. The miR-210 level of leukocytes of patients with AML was significantly higher than that of the bone marrow samples of the patients with AML. A positive correlation was found between *BCL-2* and miR-210 in bone marrow samples from patients with AML.

ÖZ

Amaç: Akut myeloid lösemi (AML), hematopoietik kök hücrelerin farklılaşmasının bozulması ile karakterize, heterojen, malign bir hastalıktır. microRNA'lar (miRNA), AML'nin oluşumunda ve ilerlemesinde etkili olan faktörlerden biridir. mikroRNA-210'un AML'nin prognozuna etkisi ve AML gelişimdeki etki ettiği yolaklar araştırılan bir konudur. *BCL-2* bir protoonkogendir ve apoptozu inhibe eden proteini kodlar. AML hücrelerinde *BCL-2*'nin aşırı ekspresyonu görülmektedir. Sitogenetik anormalliklerin varlığı veya yokluğu, AML'de önemli bir prognostik belirteçtir. Bu çalışmada, miRNA-210 ve *BCL-2* ekspresyonlarının AML prognozu üzerindeki etkisi ve sitogenetik bulgularla ilişkisinin karşılaştırılması amaçlanmıştır.

Yöntemler: AML'li hastalardan ve sağlıklı bireylerden alınan kemik iliği ve/veya periferal kan örneklerinde sitogenetik analizler gerçekleştirilmiş, RNA izolasyonunu takiben miR-210 ve *BCL-2* mRNA seviyeleri kantitatif gerçek zamanlı polimeraz zincir reaksiyonu yöntemi (qRT-PCR) ile saptanmıştır.

Bulgular: Sitogenetik analiz sonucu sağlıklı bireylerde herhangi bir anomaliye rastlanmazken, bazı AML'li hastalarda klonal yapısal ve sayısal anomaliler tespit edilmiştir. AML'li hastaların lökositlerindeki ve kemik iliği örneklerindeki *BCL-2* mRNA ekspresyon seviyelerinin sağlıklı bireylerin lökositlerindeki ekspresyon seviyelerine göre arttığı saptanırken, miR-210 seviyeleri açısından hasta ve sağlıklı bireyler arasında bir fark bulunmamıştır. Ancak AML'li hastalara ait lökositlerin miR-210 seviyesi yine

Address for Correspondence: Handenur GÖKTAN, İstanbul University-Cerrahpaşa Cerrahpaşa Faculty of Medicine, Department of Medical Biology, İstanbul, Turkey
E-mail: handenurgoktan@hotmail.com.tr **ORCID ID:** orcid.org/xxxxxxxxxxxxxxxxx

Cite this article as: Göktan H, Kuru RD, Yılmaz Ş, Alaylıoğlu M, Dursun E, Gezen Ak D, Salihoğlu A, Deviren A. mir210 and BCL2 Expressions in Patients with Acute Myeloid Leukemia.
Bezmialem Science 2022;10(4):453-60

Received: 4.12.2020
Accepted: 17.06.2021

Conclusion: The increase in *BCL-2* mRNA and miR-210 levels may have negative effects on the prognosis of the disease by causing disruption in the apoptosis mechanisms.

Keywords: AML, miRNA-210, *BCL-2*, cytogenetics, prognosis

hastaların kemik iliği örneklerindeki miR-210 seviyesinden anlamlı derecede yüksek olarak saptanmıştır. Ek olarak, AML'li hastaların kemik iliği örneklerinde *BCL-2* ve miR-210 arasında pozitif bir korelasyon bulunmuştur.

Sonuç: *BCL-2* mRNA ve miR-210 seviyelerinde görülen artış apoptoz mekanizmasında bozulmaya sebep olarak hastalığın prognozu üzerinde olumsuz etkiler yaratabilir.

Anahtar Sözcükler: AML, miRNA-210, *BCL-2*, sitogenetik, prognoz

Introduction

Acute myeloid leukemia (AML) is a hematopoietic stem cell disorder, and this malignant change occurs in hematopoietic cells, resulting in loss of function in the cells (1). The disease progresses rapidly when it is untreated and results in death according to its clinical progression (2). AML was first classified in 1976 by French-American-British classification (FAB) as M0 to M7 according to its morphological features (3,4).

According to the classification made by the World Health Organization based on cytogenetic and molecular properties, AMLs are divided into 4 groups; AML with certain genetic abnormalities, AML with myelodysplasia-related changes, AML related to previous chemotherapy or radiation, AML not otherwise specified (3,5). AML is more common in patients over 65 years of age, but it can occur at any age (6). Its incidence is approximately 3.7/100,000 per year, and the incidence of AML has increased significantly over the past decade (7).

The AML is characterized by the accumulation of over 20% myeloid blast cells in the bone marrow (8). However, in the presence of t(15;17), t(8;21), inv(16) or t(16;16) cytogenetic anomalies, AML can be diagnosed even if the number of blasts is below 20% (1). Cytogenetic findings are important parameters in the classification and prognosis of AML and chromosome anomalies are found in approximately 55% of newly diagnosed patients (9,10)

MicroRNAs (miRNA) are non-coding RNA molecules that have 19-25 nucleotides which are binding to target mRNA's 3' UTR regions and it regulates gene expression (11). Disorders of miRNA regulation affect cancer development and progression. These dysregulations cause different miRNA profiles to appear between normal and cancer tissues. The different profiles have shown that there is a relationship between cancer diagnosis and prognosis and miRNAs (12). In recent years, expressions of some specific miRNAs have been noticed to be associated with AML. miRNA-210 is also one of the miRNAs that affect prognosis of AML. Overexpression of miRNA-210 indicates poor prognosis in patients with AML (13).

The *BCL-2* protein is an inhibitor of apoptosis and is located in the outer membrane of the mitochondria, endoplasmic reticulum, and nucleus membrane (14). *BCL-2* oncogene is located on chromosome 18. This *BCL-2* locus is translocated with the immunoglobulin H locus on chromosome 14 and this translocation results in overexpression of *BCL-2* (15). Over-expression of *BCL-2* has been associated with a low complete remission rate and shorter survival after chemotherapy (16). Over-expression of *BCL-2* is associated with low treatment response in some hematological cancers such as follicular lymphoma, chronic lymphocytic leukemia, and AML (17).

In this study, it was aimed to detect chromosomal anomalies in patients with AML and to associate them with miR-210 and *BCL-2* expression levels. In this way, it would be evaluated whether miRNA-210 could be used as a biomarker with differences in treatment and prognosis.

Methods

Study Population

In this study, bone marrow materials were obtained from 7 patients who were newly diagnosed as having AML in the Cerrahpaşa Medical Faculty Hematology department and peripheral blood samples were also taken from 5 patients. The mean age of the patients was 49.4±22.4 years. Peripheral blood samples of 7 healthy volunteers (mean age was 36.8±10.6) as a control group were studied and the results were compared with the patient group. This study was approved by the Ethics Committee of İstanbul University-Cerrahpaşa, Cerrahpaşa Medical Faculty (07.09.2016-327301). Written consent form was obtained from all patients and volunteers before participating in the study.

Conventional Cytogenetics Analysis

Chromosomal analysis was performed on bone marrow samples by 24 and 48 hours cultures using standard cytogenetic technique. Seventy-two hour culture procedure was performed for peripheral blood samples (18). All samples were stained with the gas-to-liquids-banding method (19). Chromosome analysis was performed on each of the patient and control groups. The analyzes were evaluated according to The International System for Human Cytogenetic Nomenclature 2016 (20).

miRNA and mRNA Expression Analysis

The RNA was isolated from bone marrow samples of the patients by using MIRCURY Tissue RNA Isolation Kit (Exiqon, 300110). While RNA isolation from peripheral blood samples of both patients and healthy subjects was done with Qiagen RNA Isolation Kit (Qiagen, 52304). Both RNA isolation experiments were performed according to the manufacturers' protocols.

A fixed amount of RNA was fixed to 20 ng/µL for each sample for cDNA synthesis reaction and a miRCURY LNA™ microRNA PCR, Polyadenylation, and cDNA synthesis kit II (8-64 rxns) (product no. 203301, Exiqon, Denmark) were used for cDNA synthesis. miRNA expression level of miR-210 was detected by qRT-PCR using miRCURY LNA™ microRNA PCR, ExiLENT SYBR® Green master mix (Product no: 203421, Exiqon, Denmark) and LightCycler 480 instrument (Roche). UniSp6 RNA Spike-in control was used as an endogenous reference gene for normalization.

For mRNA expression analysis, cDNA synthesis was done with iScript cDNA synthesis kit (Biorad, 170-8891). From blood samples, 50 ng/µL of RNA was isolated and 150 ng/µL of RNA was isolated from bone marrow samples was used for cDNA synthesis. mRNA expression level of BCL-2 was analyzed by quantitative real-time

polymerase chain (qRT-PCR) using Universal Probe Library probe (Prob No. 75, Roche, 04688988001), Lightcycler 480 Probe Master Mix kit (Roche, 04707494001) and LightCycler 480 instrument. Actin beta (ACTB Gene Assay, Roche, 05532957001) was used as endogenous reference gene for normalization.

Statistical Analysis

The computed tomographic (CT) values obtained from qRT-PCR experiments were put into the formula ΔCT=2(reference CT - target gene CT) to determine the expression levels of both miR-210 and *BCL-2*. Microsoft Office Excel was utilized for calculations and graphics.

Raw data of each group were statistically analyzed on GraphPad InStat DTCG 3.06 software by performing Kruskal-Wallis test followed by Dunn's multiple comparisons test. $p<0.05$ was considered statistically significant. Data are presented as mean ± standard deviation.

Results

Cytogenetic Findings

The karyotype results of 7 patients with AML constituting the patient group as a result of conventional cytogenetic methods performed in bone marrow material are shown in Table 1. Clonal structural and numerical abnormalities were observed in some of the patients (Figure 1).

Peripheral blood of the control group consisting of 7 volunteers was also studied by using the conventional cytogenetic method and karyotype analysis was performed. Normal karyotype findings were seen in all volunteers.

miRNA-210 and *BCL-2* Expression Levels

The *BCL-2* mRNA expression levels in both leukocytes and bone marrrow of patients with AML were found to be increased compared to the expression levels of leukocytes of the healthy individuals ($p<0.05$ and $p<0.05$, respectively) (Figure 2).

The miR-210 expression level of leukocytes of patients with AML was higher than the level of bone marrow of the patients with AML ($p<0.05$). Yet, there was no difference between patients and healthy individuals ($p>0.05$) (Figure 3). It was important to note that one patient was not included in statistical analysis given the low levels of miR-210 expression (cycle threshold was higher than 35 in qRT-PCR).

A correlation analysis was also performed between *BCL-2* and miR-210 expression levels. A positive correlation between *BCL-2* and miR-210 was found in bone marrow samples of patients with AML ($r2=0.89$; 95% confidence interval: 0.77-0.99; $p<0.0001$) (Figure 4).

Discussion

Dysregulation of miRNA regulation leads to disruption in the hematopoietic system and can cause leukemia (21). It has been supported by studies that miRNA-210 plays a role as a tumor suppressor or oncomir according to cancer type (22). Most studies have shown that high miR-210 levels in cancerous tissues are associated with poor prognosis (23-27). However, there are also studies showing opposite results (23,24,28-30).

In the literature, a single study has been found showing the expression of miR-210 in patients with AML. In this study, the level of miR-210 expression in the peripheral blood and bone marrow material of patients with AML was investigated. In the findings obtained, overexpression of miR-210 in patients with AML in both serum and bone marrow was compared with the healthy control group. There was no relationship between serum miR-210 level and age, sex, white blood cell amount and complete remission. It was found to be related to cytogenetic findings and FAB classification. In addition, it was found that the overall survival rate was worse and the survival rate without relapse was worse in patients with high miR-210 levels. The findings showed that miR-210 might be a prognostic marker for AML (13).

In our study, the level of miR-210 expression detected from peripheral blood of patients with AML was found to be significantly higher than the expression level detected from bone marrow, yet there was no difference between patients and healthy individuals. This result is not fully compatible with the literature. A reason for this may be due to the low number of patients.

The *BCL-2* is classified as an oncogene (31). Ongoing studies show that *BCL-2* damage causes cell death. It is also known that *BCL-2* plays a role in developing resistance to chemotherapeutic agents (32).

In AML, over-expression of *BCL-2*, resistance to chemotherapy and low overall survival rates were first investigated in a study by Tóthová et al. (16). Data from another study conducted in later years shed some points light on *BCL-2*'s expression and its role in disease. In most of the patients studied, *BCL-2* expression was significantly higher and was associated with poor clinical prognosis and poor response after intense chemotherapy. High *BCL-2* expression level supports the hypothesis that the apoptotic

Table 1. Patients age, gender, FAB classification, and results of conventional cytogenetic analysis

Patient	Age	Sex	Diagnosis	Karyotyping
1	24	M	AML M2	46,XY[4] *NCA[2]
2	21	M	AML M4	41~44,XY,-18[3],-21[3],-22[3][cp6]/46,XY[5]
3	62	M	AML M4	40~47,XY,+4[2],-5[3],-9[3],inv(9)(p11q13)[8],add(21)(p11)[3],-22[3],+mar1[3],+mar2[2] [cp9]/46,XY,inv(9)(p11q13)[10]**
4	52	F	AML M5	***-
5	42	F	AML	46,XX[2] *NCA[3]
6	61	M	AML	25~27,X,+2[3],+5[2],+9[2],+12[2],+20[2][cp3]/35~44,XY,-6[4],-8[4],-9[3],-21[3] [cp6]/46,XY[7]
7	84	M	AML	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]

*non-clonal abnormalities, **inv(9)(p11q13) finding is polymorphic, ***No quality metaphase to evaluate,
FAB: French-American-British classification, AML: Acute myeloid leukemia

mechanism contributes to tumorigenesis. In addition, anomaly in *BCL-2* protein has been shown to cause longer survival of cancerous cells, thereby negatively affecting drug resistance and chemotherapy response with increased white blood cells. And also, data obtained as a result of experiments suggested that the functional role of *BCL-2* was to block apoptosis without affecting cell proliferation (33-35). In our study, we found that mRNA expression level of *BCL-2* in bone marrow samples of newly diagnosed patients with AML was found to be significantly higher than the levels in peripheral blood samples of healthy subjects. In addition, the level of *BCL-2* expression in peripheral blood from patients was significantly increased compared to the healthy individuals. The data we obtained are compatible with the data in the literature. Compared to the level of *BCL-2* expression in bone marrow and peripheral blood, *BCL-2* expression in the bone marrow was found to be higher. This may be due to the high expression of *BCL-2* in tissues with more apoptosis, such as bone marrow (36). In our patients, higher *BCL-2* expression in the bone marrow is compatible with the literature in this regard.

Among the target genes of miR-210, when the databases showing miRNA target genes were examined, *BCL-2* could not be found. In our study, it was investigated whether miR-210 and *BCL-2* levels showed correlation in AML. When the studies on this subject in the literature were examined, a study supporting the increase theory was found. Data obtained in the study of neurons in cell culture and PC12 cell line in mice showed that over-expression of miR-210 suppressed apoptosis in a correlation with increased *BCL-2* level (37). The data we obtained in our study showed that *BCL-2* expression and miR-210 expression in the bone marrow of patients with AML were positively correlated. The increase of miR-210 and hence *BCL-2* confirms the theory that leukemia cells increase by suppressing apoptosis in AML, and therefore causing disease deterioration. This findings may indicate that *BCL-2* is not a target of miR-210 and miR-210 may regulate apoptosis through other targets, such as HIF-1-a (38). More comprehensive studies are needed to clarify this mechanism more clearly.

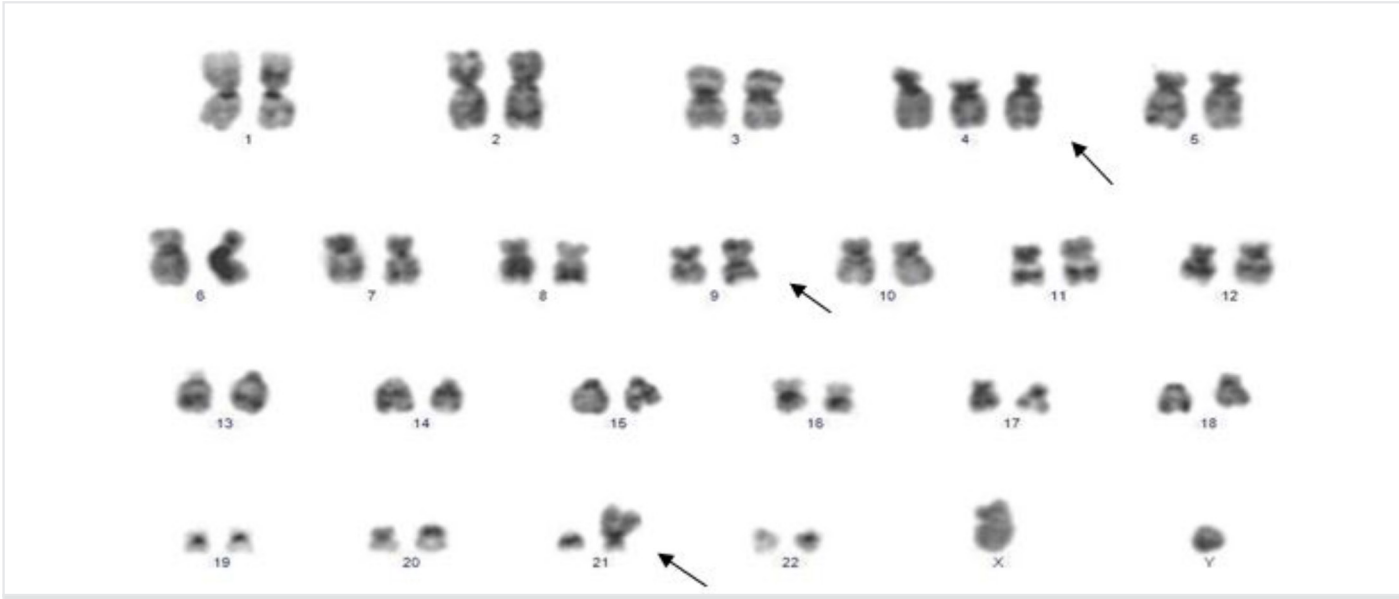

Figure 1. Karyotype analysis of patient 3 including numerical and structural chromosomal abnormalities.
Karyotype: *47,XY,+4,inv(9)(p11q13),add(21)(p11)*

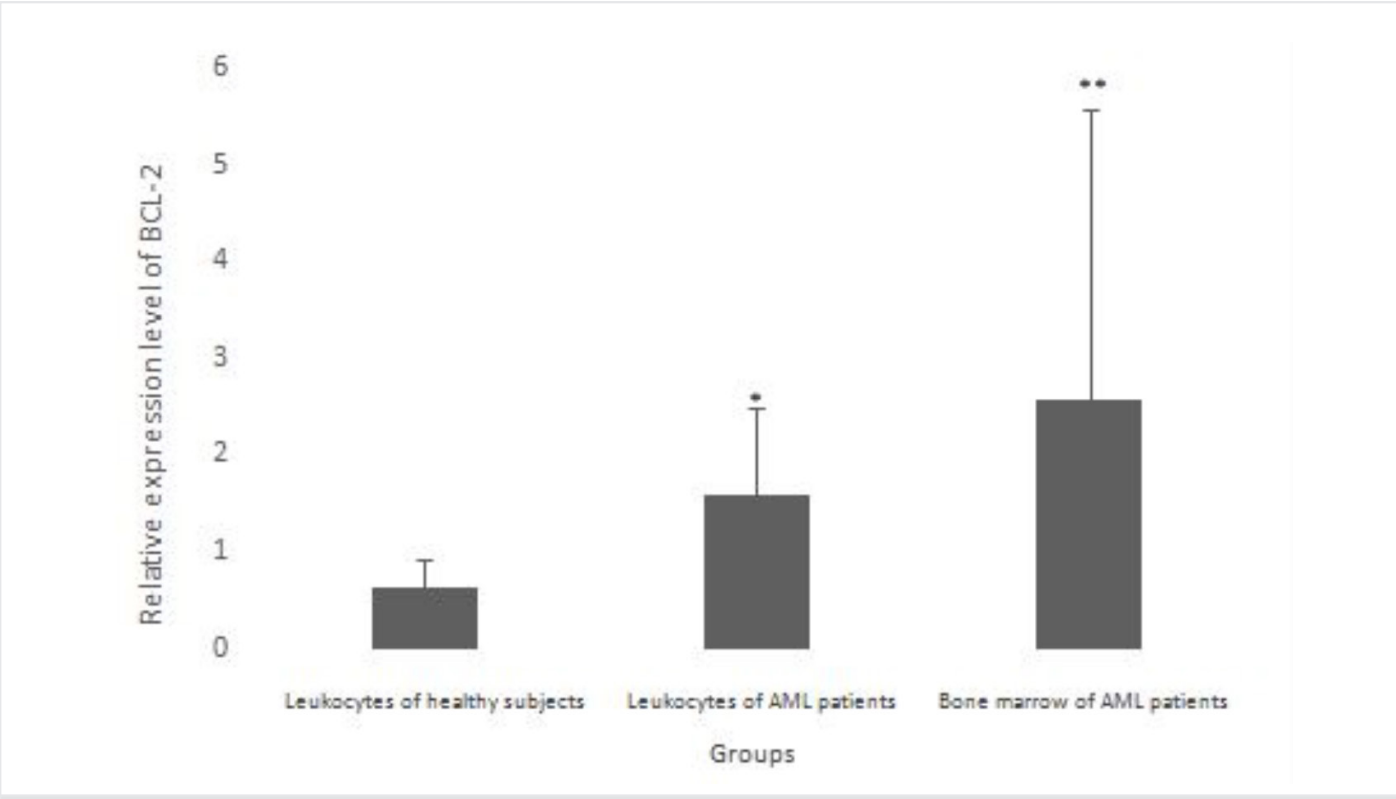

Figure 2. BCL-2 mRNA expression levels of patients with AML and healthy individuals
AML: Acute myeloid leukemia

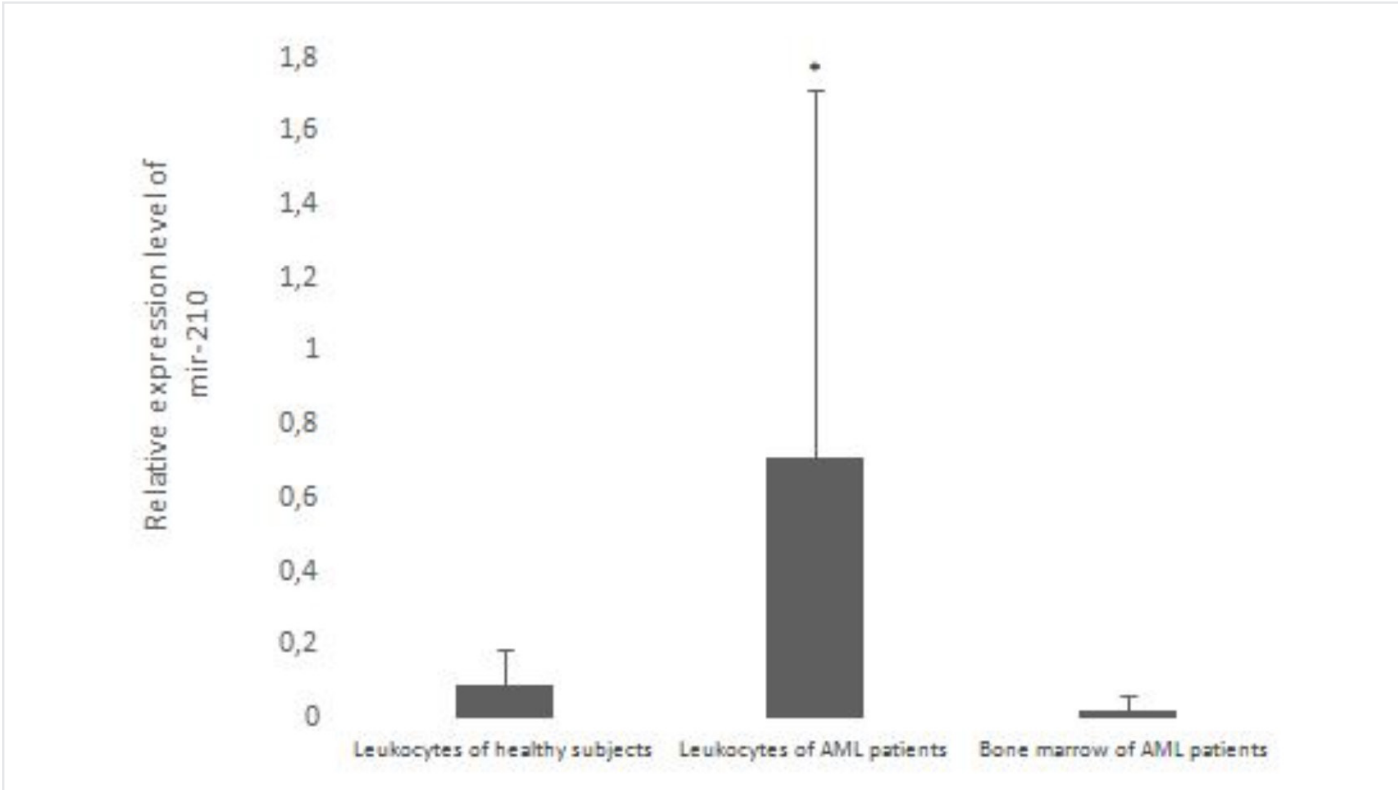

Figure 3. mir-210 expression level of patients with AML and healthy individuals
AML: Acute myeloid leukemia

Cytogenetic findings play an important role in the prognosis of AML. In a study of patients with AML, the presence of only trisomy 21 (+21) or the presence of +21 with additional anomalies was found to be associated with poor prognosis. A poor prognosis is predicted especially in patients with AML over 60 years old with only +21 (39). In our study, clonal +21 findings were found in one patient. Also, other numerical chromosomal anomalies were seen in this patient. Although our findings were consistent with the literature, the age of our patient was 84, which supported the prediction of poor prognosis in patients over 60 years of age.

In the bone marrow material of 3 patients examined in our study, monosomy 21 (-21) was found clonally. However, the finding of -21 was accompanied by several numerical chromosomal anomalies. In the literature, there were patients in whom only -21 was seen in AML and other hematological diseases (such as multiple myeloma and myelodysplastic syndrome (MDS) (40). In an article, it was predicted that the loss or increase of chromosome 21 in MDS would cause a moderate prognosis (41). MDS is a hematological disease that has the potential to transform into AML. Therefore, the -21 anomaly seen in the patients examined in our study may be significant in terms of prognosis.

According to the data obtained from the literature, patients with monosomal karyotype and complex karyotype are associated with poor prognosis. Patients who have a poor prognosis or complex karyotype have a worse prognosis than patients who have a monosomy karyotype. According to the data obtained from previous studies, -5, -7, -8, -17, -21, -22 are the most commonly seen monosomies (42). In our study, monosomies of chromosomes -5, -8, -21 and -22 were observed in some patients. In these patients, both the monosomal karyotype and complex karyotype were detected. Especially in one patient, the disease progressed very shortly after diagnosis, resulting in hospitalization and death. In patient 6, the monosomal karyotype (monosomy 8 was also present) and complex karyotype findings, which were indicative of poor prognosis, were seen together. At the same time, because our patient was over 60 years old, it was compatible with the thesis that poor prognosis in patients with AML over 60 years old.

Study Limitations

We encountered some limitations during our study. One of them was to find a patient with *de novo* AML who had not yet received treatment. For this reason, we could not reach the target number of patients. Another limitation was the lack of sufficient financial resources to perform apoptotic tests. Therefore, additional studies are needed to support our apoptosis theory. The last limitation was that healthy bone marrow samples could not be part of our study due to the ethical reasons. For this reason, *BCL-2* and miR-210 expression levels in bone marrow samples of patients with AML were compared with the leukocytes of healthy volunteers instead of bone marrow samples.

Conclusion

As a result, in our study, increased expression levels of miR-210 and *BCL-2* were observed in the bone marrow material in AML, and findings indicating that this increase occurred in a correlation were obtained. It was concluded that this increase might cause disruption in the apoptosis mechanism and might have negative effects on prognosis. In addition, as a result of the data we obtained, we believe that the expression of miR-210 and *BCL-2* can be useful as a biomarker for AML disease. In addition, we think that the cytogenetic findings obtained will be beneficial in terms of prognosis evaluation. We believe that the effects of miR-210 and *BCL-2* on the apoptosis pathway, especially in AML, should be investigated and a larger number of samples and comprehensive tests are needed to clarify this mechanism and obtain precise results.

Ethics

Ethics Committee Approval: This study was approved by the Ethics Committee of İstanbul University-Cerrahpaşa Cerrahpaşa Faculty of Medicine (07.09.2016-327301).

Informed Consent: Written consent form was obtained from all patients and volunteers before participating in the study.

Peer-review: Externally and internally peer reviewed.

Authorship Contributions

Concept: H.G., R.D.K., Ş.Y., Design: H.G., R.D.K., Ş.Y., M.A., Data Collection or Processing: H.G., E.D., D.G.A, A.S., A.D., Analysis or Interpretation: H.G., R.D.K., Ş.Y., M.A., E.D., D.G.A, A.S., A.D., Literature Search: H.G., R.D.K., Ş.Y., M.A., E.D., Writing: H.G., R.D.K., Ş.Y., M.A., E.D., D.G.A, A.S., A.D.

Conflict of Interest: All authors have declared that they no conflict of interest.

Financial Disclosure: The present work was supported by the Research Fund of İstanbul University-Cerrahpaşa (project no: TYL-2016-22771)

References

1. Shipley JL, Butera JN. Acute myelogenous leukemia. Exp Hematol 2009;37:649-58.
2. Estey E, Döhner H. Acute myeloid leukaemia. Lancet 2006;368:1894-907.
3. Fortina P, Londin E, Park JY, Kricka LJ. Acute myeloid leukemia : methods and protocols. 2017.
4. WHO. ACUTE MYELOGENOUS LEUKEMIA (Including Acute Promyelocytic Leukemia). 2014 Rev Cancer Med 2014;(List of Essential Medicines):1-14.
5. Mughal T. Understanding Leukemias, Lymphomas and Myelomas [Internet]. CRC Press; 2005. Available from: https://books.google.com.tr/books?id=Z0UqBgAAQBAJ
6. Ferrara F, Schiffer CA. Acute myeloid leukaemia in adults. Lancet 2013;381:484-95.
7. Longo DL. Harrison's hematology and oncology. New York: McGraw-Hill Medical; 2010.
8. Estey EH, Faderl SH, Kantarjian HM. Hematologic Malignancies: Acute Leukemias [Internet]. Springer Berlin Heidelberg; 2007.

(Hematologic Malignancies). Available from: https://books.google.com.tr/books?id=im1Fdb19geUC

9. Yohe S. Molecular Genetic Markers in Acute Myeloid Leukemia. J Clin Med 2015 ;4:460-78.
10. Mrózek K, Heerema NA, Bloomfield CD. Cytogenetics in acute leukemia. Blood Rev 2004 ;18:115-36.
11. Gao HY, Wang W, Luo XG, Jiang YF, He X, Xu P, et al. Screening of prognostic risk microRNAs for acute myeloid leukemia. Hematology 2018;23:747-55.
12. Harrandah AM, Mora RA, Chan EKL. Emerging microRNAs in cancer diagnosis, progression, and immune surveillance. Cancer Lett 2018;438:126-32.
13. Tang X, Chen L, Yan X, Li Y, Xiong Y, Zhou X. Overexpression of miR-210 is associated with poor prognosis of acute myeloid leukemia. Med Sci Monit 2015;21:3427-33.
14. Kale J, Osterlund EJ, Andrews DW. BCL-2 family proteins: changing partners in the dance towards death. Cell Death Differ 2018;25:65-80.
15. Irvine AE, McMullin MF, Ong YL. Bcl-2 Family Members As Prognostic Indicators in AML. Hematology 2002;7:21-31.
16. Tóthová E, Fricova M, Stecová N, Kafková A, Elbertová A. High expression of Bcl-2 protein in acute myeloid leukemia cells is associated with poor response to chemotherapy. Neoplasma 2002;49:141-4.
17. Wojcik I, Szybka M, Golanska E, Rieske P, Blonski JZ, Robak T, et al. Abnormalities of the P53, MDM2, BCL2 and BAX genes in acute leukemias. Neoplasma 2005;52:318-24.
18. Waters J. Human Cytogenetics: A Practical Approach, Volume II: Malignancy and Acquired Abnormalities (second edition). Edited by D. E. Rooney and B. H. Czepulkowski. IRL Press at Oxford University Press. 1992. 293 pages. Price Paper £22.50, ISBN 0 19963313 4. Spira. Genet Res [Internet]. 2009/04/14. 1993;61(1):76. Available from: https://www.cambridge.org/core/article/human-cytogenetics-a-practical-approach-volume-ii-malignancy-and-acquired-abnormalities-second-edition-edited-by-d-e-rooney-and-b-h-czepulkowski-irl-press-at-oxford-university-press-1992-293-pages-price-paper-2250-is
19. Therman E. Human Chromosomes: Structure, Behavior, Effects [Internet]. Springer-Verlag; 1980. Available from: https://books.google.com.tr/books?id=vMlqAAAAMAAJ
20. McGowan-Jordan J, Simons A, Schmid M. ISCN 2016: An International System for Human Cytogenomic Nomenclature (2016) [Internet]. Karger; 2016. (An International system for human cytogenetic nomenclature). Available from: https://books.google.com.tr/books?id=0y3JjwEACAAJ
21. Mardani R, Jafari Najaf Abadi MH, Motieian M, Taghizadeh-Boroujeni S, Bayat A, Farsinezhad A, et al. MicroRNA in leukemia: Tumor suppressors and oncogenes with prognostic potential. J Cell Physiol 2019;234:8465-86.
22. Huang X, Le QT, Giaccia AJ. MiR-210--micromanager of the hypoxia pathway. Trends Mol Med 2010;16:230-7.
23. Camps C, Buffa FM, Colella S, Moore J, Sotiriou C, Sheldon H, et al. hsa-miR-210 Is Induced by Hypoxia and Is an Independent Prognostic Factor in Breast Cancer. Clin Cancer Res 2008;14:1340-8.
24. Gee HE, Camps C, Buffa FM, Patiar S, Winter SC, Betts G, et al. hsa-mir-210 is a marker of tumor hypoxia and a prognostic factor in head and neck cancer. Cancer 2010;116:2148-58.
25. Ho AS, Huang X, Cao H, Christman-Skieller C, Bennewith K, Le QT, et al. Circulating miR-210 as a Novel Hypoxia Marker in Pancreatic Cancer. Transl Oncol 2010;3:109-13.
26. Zhao A, Li G, Péoc'h M, Genin C, Gigante M. Serum miR-210 as a novel biomarker for molecular diagnosis of clear cell renal cell carcinoma. Exp Mol Pathol 2013;94:115-20.
27. Qiu S, Lin S, Hu D, Feng Y, Tan Y, Peng Y. Interactions of miR-323/miR-326/miR-329 and miR-130a/miR-155/miR-210 as prognostic indicators for clinical outcome of glioblastoma patients. J Transl Med 2013;11:10.
28. Giannakakis A, Sandaltzopoulos R, Greshock J, Liang S, Huang J, Hasegawa K, et al. miR-210 links hypoxia with cell cycle regulation and is deleted in human epithelial ovarian cancer. Cancer Biol Ther 2008;7:255-64.
29. Lawrie CH, Gal S, Dunlop HM, Pushkaran B, Liggins AP, Pulford K, et al. Detection of elevated levels of tumour-associated microRNAs in serum of patients with diffuse large B-cell lymphoma. Br J Haematol 2008;141:672-5.
30. McCormick RI, Blick C, Ragoussis J, Schoedel J, Mole DR, Young AC, et al. miR-210 is a target of hypoxia-inducible factors 1 and 2 in renal cancer, regulates ISCU and correlates with good prognosis. Br J Cancer 2013;108:1133-42.
31. Reed JC. Bcl-2-family proteins and hematologic malignancies: history and future prospects. Blood 2008;111:3322-30. Erratum in: Blood 2008;112:452.
32. Thomas S, Quinn BA, Das SK, Dash R, Emdad L, Dasgupta S, et al. Targeting the Bcl-2 family for cancer therapy. Expert Opin Ther Targets 2013;17:61-75.
33. Zhou J, Chen Y, Li C, Liu W. The protein expression of Bcl-2, Bax, Fas/Apo-1 in acute myeloid leukemia. J Tongji Med Univ 1998;18:42-5.
34. Del Poeta G, Venditti A, Del Principe MI, Maurillo L, Buccisano F, Tamburini A, et al. Amount of spontaneous apoptosis detected by Bax/Bcl-2 ratio predicts outcome in acute myeloid leukemia (AML). Blood 2003;101:2125-31.
35. Mehta S V, Shukla SN, Vora HH. Overexpression of Bcl2 protein predicts chemoresistance in acute myeloid leukemia: its correlation with FLT3. Neoplasma 2013;60:666-75.
36. Mehta S V, Shukla SN, Vora HH. Overexpression of Bcl2 protein predicts chemoresistance in acute myeloid leukemia: its correlation with FLT3. Neoplasma 2013;60:666-75.
37. Qiu J, Zhou XY, Zhou XG, Cheng R, Liu HY, Li Y. Neuroprotective effects of microRNA-210 against oxygen-glucose deprivation through inhibition of apoptosis in PC12 cells. Mol Med Rep 2013;7:1955-9.
38. Chio CC, Lin JW, Cheng HA, Chiu WT, Wang YH, Wang JJ, et al. MicroRNA-210 targets antiapoptotic Bcl-2 expression and mediates hypoxia-induced apoptosis of neuroblastoma cells. Arch Toxicol 2013;87:459-68.

39. Lu Y, Yuan J, Wang H, Pei R, Chen Z, Jin J. [Clinical features and prognosis of acute myeloid leukemia with acquired trisomy 21]. Zhonghua yi xue yi chuan xue za zhi = Zhonghua yixue yichuanxue zazhi = Chinese J Med Genet 2017;34:554-8.
40. Freitas PC, Carvalho-Salles AB, Mendiburu CF, Ricci OJ, Fett-Conte AC. Clonal monosomy of chromosome 21 in a case of myelodysplastic syndrome. Genet Mol Res 2011;10:2718-20.
41. Bacher U, Schanz J, Braulke F, Haase D. Rare cytogenetic abnormalities in myelodysplastic syndromes. Mediterr J Hematol Infect Dis 2015;7:e2015034.
42. Li Z, Wei H, Lin D, Zhou CL, Liu BC, Wang Y, et al. Cytogentic and prognostic characteristic of acute myeloid leukemia with monosomal karyotype. Zhonghua Xue Ye Xue Za Zhi 2016;37:366-71.