MicroRNA Expression Profiles and Changes with Treatment on Childhood Leukemias

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ABSTRACT

MicroRNAs are non-protein coding RNAs that have recently been revealed to be effective in cancer biogenesis which effect post-transcriptional gene modification. The incidence of childhood hematologic cancers is increasing. Therefore, this study aimed to investigate the relationship between miRNA expression and its prognosis in childhood leukemias. Twenty-one patients and 5 healthy control subjects were included in the study. Out of 26 subjects, 15 were patients diagnosed with acute lymphoblastic leukemia (ALL), 6 were patients diagnosed with acute myeloblastic leukemia (AML) and 5 were healthy control subjects. Peripheral venous samples were collected from the patients before and 1 month after chemotherapy and their miRNA analyses were performed. Decreased expressions of let-7b, miR-31, miR-128-1, miR-218, miR-331, miR-372, miR-375, miR-422, miR-451 and miR-520 were found in patients with AL compared to the healthy control subjects. While the expression of miR-375 decreased in patients with ALL, the expressions of miR-21, miR-222, miR-30, miR-145, miR-146a and miR-155 increased. While the expression of miR-155 increased in patients with AML, the expressions of miR-10, miR-23, miR-218, miR-422 and miR-451 decreased. It was also found that while the expressions of miR-204,miR-7,miR-10, miR-30, miR-155, miR-192, miR-422, miR-451,miR-520, miR-548, miR-375, miR-1, miR-23 and miR-146a increased in patients with leukemia after the treatment, the expressions of let-7b and miR-132 decreased. A positive correlation was found between leukocyte count of patients with leukemia before the treatment and the expressions of miR-128-1 and miR-331 among miRNAs that changed after the treatment. MiRNAs play role in the pathogenesis, treatment response, relapse and prognosis of hematologic malignancies.

Keywords: microRNA, Childhood leukemias

ÖZET

Çocukluk Çağı Lösemilerinin Mikrorna Profili ve Tedavi İle Değişimi

MikroRNA'lar, son zamanlarda kanser biyogenezinde etkili oldukları gösterilmiş, protein kodlamayan RNA'lardır. Etkilerini transkripsiyon sonrası gen modifikasyonu ile göstermektedirler. Çocukluk çağı hematolojik kanseri sıklığı giderek artmaktadır. Bu nedenle çalışmamızda çocukluk çağı hematolojik malignitelerinde miRNA ekspresyonu ve prognoz ile ilişkisini araştırılması amaçlanmıştır. Çalışmaya 26 hasta ve 5 sağlıklı kontrol alındı. Hastaların 15'i akut lenfoblastik lösemi, 6'sı akut myeloblastik lösemi tanısı almış hastalardan ve 5 sağlıklı kontrolden oluşmaktaydı. Kontrol grubunu yaş ortalaması 9.2 ±2.2 yıl olup 3'ü erkek (%60), 2'si kızdı (%40). Akut lösemi hastalarının dokuzu (%42.9) kız, 12'si (%57.1) erkekti. Akut lösemi hastalarının ortalama yaşı 8,5 ±7,2 yıl, ortanca yaşı 5,7 yıl (1-27) olarak saptandı. Hastalardan kemoterapi öncesi ve tedavinin 1 ayı tamamlandıktan sonra periferik venöz örnek alınarak miRNA analizleri yapıldı. Akut lösemi hastalarında sağlıklı kontrollere göre let-7b, mir-31, miR-128-1, miR-218, miR-331, miR-372, miR-375, miR-422, miR-451 ve miR-520 ekspresyonları azalmış bulundu. ALL hastalarında miR-375 ekspresyonunun azaldığı, miR-21, miR-222, miR-30, miR-145, miR-146a ve miR-155 ekspresyonunun arttığı saptandı. AML hastalarında miR-155'in ekspresyonu artarken, miR-10, miR-23, miR-218, miR-422, miR-451'in ekspresyonunun azaldığı görüldü. Lösemi hastalarında tedavi sonrası miR-204, miR-7, miR-10, miR-30, miR-155, miR-192, miR-422, miR-451, miR-520, miR-548, miR-375, miR-1, miR-23 ve miR-146a'nın ekspresyonlarının arttığı; let-7b ve miR-132'nin ekspresyonlarının azaldığı saptandı. Lösemi hastalarının tedavi öncesi lökosit sayıları ile tedaviden sonra değişen miRNA'lardan miR-128-1 ve miR-331 ile lökosit sayısı arasında pozitif yönde ilişki bulundu Sonuç olarak miRNA'lar hematolojik malignitelerin patogenezinde, tedaviyanıtında, relaps ve prognozunda rol oynamaktadırlar.

Anahtar Kelimeler: mikroRNA, Çocukluk çağı lösemileri

INTRODUCTION

Childhood cancers are a group of cancers developing under the age of 15 and consist of 0.5-4.6% of cancers. The worldwide incidence of cancer in children ranges from 50 to 200 in a million.[1,2] While leukemia (34%) constitutes the majority of childhood cancers, it was followed by central nervous system (CNS) tumors (23%), lymphomas (12%) and soft tissue-bone sarcomas. In the study of Turkish Pediatric Oncology group with more than 60 centers in our country, leukemia cases constitute 30.33% of pediatric cancer records and are followed by lymphomas with a rate of 18.89%. When the distribution of leukemia cases was evaluated, the incidence of lymphoid leukemia was the highest with a rate of 78.81% and followed by acute myeloid leukemia with a rate of 16.97% and the third highest incidence rate belonged to chronic myeloid leukemia with a rate of 1.85%.[3]

MicroRNAs are functional RNA molecules that are transcribed by protein-coding intron and exon regions on genome and non-protein-coding RNA genes but that are not translated into proteins. MiRNA is a type of RNA molecule that functionally plays a role in gene expression regulation and that is approximately 22-nucleotides in length and single-stranded. Hundreds of highly conserved regions of gene coding miRNAs in human genome were discovered. So far, more than 1000 miRNAs were defined.[4] The first miRNA was discovered by Lee et al. in 1993 and the concept of "microRNA" started to be used by 2001. The first miRNA, lin-14, discovered in a nematode called Caenorhabditis elegans was found to be functioning by binding to mRNA of lin 14 gene in the site of 3' UTR.[5,6]

Mature miRNAs join the regulation of protein synthesis by decreasing the expression level of target genes. MicroRNAs are able to recognize target genes complementary to their nucleotide sequences. A microRNA binds to mRNA by forming a complex with R S and causes the inhibition of translation or mRNA degradation.[7] The binding type of microRNA to the target mRNA results in mRNA degradation or inhibition of translation. The reason is that if a miRNA binds to the untranslated region (3' UTR) of the target mRNA, this results in incomplete complementarity and translational repression. However, if there is complementarity, miRNA binds to the 'open reading frame' region of the target mRNA and mRNA degradation occurs by Argonaut 2.[8] While each miRNA regulates more than one mRNA expression, it was found that mRNAs could be targeted by more than one miRNA.[9]

The fact that miRNAs take a role in several processes such as cell proliferation and apoptosis suggests that they are effective in cancer biogenesis.[10] They were also revealed to be associated not only with the pathogenesis of cancer but also with inflammatory diseases, cardiac diseases and glomerular diseases.[11-13]

The first study in which the effect of miRNAs on canceration process was conducted by Alin et al. in 2001. In this study, as deletion was seen in the gene location of 13q14 in about half of the patients with chronic lymphocytic leukemia (CLL), miRNA-15 and miRNA-16 located on the same gene were found to be downregulated in most (68%) of the patients with CLL.[14] When miRNA analyses of the subgroups in pediatric patients with ALL were evaluated, it was found that the expressions of miR-148, miR-151 and miR-424 could be used in the distinction of B and T cell ALL.[15]

Although prognostic value of chromosomal changes in leukemia is well studied previously16, the relationship of miRNAs and clinical characteristics of patients diagnosed with leukemia was not clear. In our study, we aimed to evaluate miRNA expression profiles of children diagnosed with leukemia and relationship with their clinical characteristics.

PATIENTS AND METHODS

This study was performed with patients who were newly diagnosed with acute leukemia at Pamukkale University Faculty of Medicine Hospital between August 2014 and September 2015. Ethical approval was obtained from the Ethical Committee of Pamukkale University, Faculty of Medicine (decision number of 11 dated 13.08.2013). Moreover, informed consent forms were obtained from patients and their parents and the control group. Patients between the ages of 0-25 who were newly diagnosed with acute leukemia were included in the study. Patients whose therapies had already been

initiated, who had any inherited haematological disease, who developed acute leukemia secondary to the genetic syndrome and patients whose consents to attend the study were not obtained were excluded from the study.

Venous blood samples of 2.5 mL were collected from the patients included in the study both at the time of diagnosis and when the one month of the therapy was completed. As the level of RNA would start to rapidly decrease because of its chemical structure, the samples were kept in special tubes containing a chemical to stabilize the level of RNA. By doing this, we aimed to keep the levels of RNA stabilized while transferring the samples from the place where they were collected to the laboratory where they would be isolated. In this study, PAXgen tubes were used as stabilizers. The structure and amount of RNA in these tubes can be stabilized up to 3 days at 18-25^{0}C and 5 days at 2-8^{0}C. They can be stored for approximately 50 months at -70^{0}C.

The stages of the study were as follows: plasma extraction from peripheral venous blood samples, RNA isolation, sDNA synthesis, and expression analysis of qRT-PCR miRNA. Qiagen miRNeasy kit (catalog no:217184, Germany) was used for RNA isolation. miRNA target genes were determined by using miRGene target database (http://www.diana.pcbi.upenn.edu/cgi-bin/miRGen/v3/Targets.cgi). For real time reaction, obtained cDNAs were used. U6 was used as normalizer. Differences in the expression levels of patients' microRNAs before and after the therapy were determined with bioinformatics support by analyzing qRT-P R results.

t values obtained in the study were analysed for the statistical analysis of qRT-P R studies and delta t analysis was used for fold change analysis. Differences among the groups were calculated with the formula of 2-ΔΔCT = [(ΔCT(patient) – ΔCT(u6) – (ΔCT(control) – Δ T(u6)]. SPSS 20.0 package software was used in data analysis. Descriptive statistics were used for the characteristics of the patients in the study and Mann-Whitney U test was used for the comparison of the miRNA expression differences between the patients with ALL and AML and the control group. Kruskal-Wallis analysis was used for the miRNA expression differences in ALL patients who were classified according to the risks. The relationship between leukocyte count and percentage of blast cells on peripheral smear and in bone morrow and the change in the expressions of miRNAs was evaluated with Spearman's correlation test. The significance level was determined as $p< 0.05$ in all the tests.

RESULTS

Twenty-one patients and 5 healthy control subjects were included in the study. Healthy children without any chronic disease were included in the control group. The mean age of the control group was 9.2±2.2 years and while 3 of them were male (60%), 2 were female (40%). Out of the patients with acute leukemia, 9 (42.9%) were female and 12 (57.1%) were male. The mean age of the patients with acute leukemia was 8.5±7.2 years and their median age was 5.7 years (min: 1 – max: 27). Two of the patients with ALL were in the age group of adults. Out of patients with leukemia, 15 (71.4%) had ALL while 6 (28.6%) had AML. Out of 15 patients with acute lymphoblastic leukemia, 2 (13%) had T-cell ALL while 13 (86.6%) had B-cell ALL. According to the French-American-British (FAB) classification system, 5 of the patients with ALL were subtyped as L1, 3 patients as L2, and 1 patient as L3. FAB classifications of 6 patients were not stated. According to FAB classification, 3 of the patients with AML were subtyped as M2, 2 patients as M4, and 1 patient as M0. inv(16) positivity was detected in one and t(8:21) positivity was detected in one of the patients with AML. When the patients were divided into the risk groups according to the Berlin-Frankfurt-Munster (BFM) protocol, 4 (26.6%) of the patients with ALL were in the standard-risk group, 8 (53.3%) in the intermediate-risk group and 3 (20%) in the high-risk group. Four (66.6%) of the patients with AML were in the low-risk group while two (33.3%) were in the high-risk group. While all the patients with ALL responded to steroid therapy on the 8th day, only 1 patient did not go into remission on the 33rd day (4.8%). All the patients with AML went into remission in one month. While mean initial leukocyte (WBC) count of the patients was 29.535±32.499/mm^3 during presentation and mean percentage of blast cells on

Table 1. Demographic, clinical and laboratory profiles of the patients with acute leukemia

Patient number	Age (year)	Sex	Subtype	Immuno-phenotype	FAB classi-fication	Cytogenetics	Risk group	Remission status
1	3	F	ALL	B-cell	L1	t(12:21)	Standard	In remission
2	22	M	ALL	B-cell		-	High	Exitus
3	6	F	ALL	B-cell	L1	t(12:21)	Intermediate	In remission
4	5	M	AML		M0	-	High	In remission
5	4,5	F	AML		M4	Inv 16	Low	In remission
6	13	F	AML		M2	t(8:21)	Low	In remission
7	4	M	ALL	B-cell	L3	-	High	Relapse
8	27	F	AML		M2	-	Low	In remission
9	13	M	ALL	B-cell	L2	-	Intermediate	In remission
10	2	M	ALL	B-cell	L1	-	Standard	In remission
11	13,5	M	ALL	B-cell	L2	-	Intermediate	In remission
12	4	M	ALL	T- cell		-	Intermediate	In remission
13	14	M	ALL	T-cell		-	High	Not in remission
14	5	M	ALL	B-cell		-	Standard	In remission
15	1	F	ALL	B-cell		-	Intermediate	Out of follow-up
16	7	M	ALL	B-cell		Hyperdiploidy	Intermediate	In remission
17	2	M	AML		M4	t(4:11)	High	In remission
18	16	M	AML		M2	-	Low	In remission
19	2	F	ALL	B-cell	L1	-	Standard	In remission
20	14	F	ALL	B-cell	L1	-	Intermediate	In remission
21	2	F	ALL	B-cell	L2	-	Intermediate	In remission

F= female; M= male; ALL= acute lymphoblastic leukemia; AML= acute myeloblastic leukemia

peripheral smear was 75.9% (12-100), mean percentage of blast cells in bone marrow was 85.1% (16-100). Philadelphia chromosome was negative in all of the patients with ALL. Hyperdiploidy was detected in one and t(12:21) in two patients. Demographic and clinical characteristics of the patients included in the study were given in Table 1.

Expression levels of 33 miRNAs in patients were examined before the treatment and in the 1st month of the treatment. The examined miRNAs were selected from the ones that were revealed to play role in the pathogenesis of cancer in literature. These miRNAs were miR-1, let-7b, miR-7, miR-10, miR-21, miR-222, miR23, miR-27a, miR-25, miR-30, miR-31, miR-132, miR-135b, miR-128-1, miR-145, miR-146a, miR-150, miR-155, miR-181, miR-192, miR-200b, miR-200c, miR-204, miR-218, miR-331, miR-371, miR-375, miR-422, miR-451, miR-494, miR-499, miR-520, and miR-548. U6 was used for standardization. Comparison of miRNA levels in patients with acute leukemia with those in the control group and calculation of how much they changed before and after the therapy was performed with "fold change analysis". MiRNA fold change analyses were given in Table 2. In the comparison of the patients with acute leukemia with healthy control subjects, the expressions of miR-21, miR-222, miR-30, miR-145, miR-146a, and miR-181 were statistically significantly high while those of let-7b, miR-128-1, miR-218, miR-331, miR-422, miR-451, miR-520, miR-31, miR-372, and miR-375 were significantly low. miRNA fold change analyses of acute leukemia and control groups were given in Table 2. Compared to the control group, while the expression of miR-155 increased and the expressions of miR-10 miR-23, miR-218, miR-422, and miR-451 decreased in patients with AML, the expressions of miR-21, miR-222, miR-30, miR-145, miR-146a,

Table 2. Comparison of miRNA fold levels of patients with leukemia and control group

MiRNA		Median (Min - Max)	p	MiRNA		Median (Min - Max)	p
miR1	leukemia (n=21)	12.57 (0 - 18458.53)	0.659	miR146a	leukemia (n=21)	2234.91 (0.01 - 704.38 x10^3)	0.001*
	control (n=5)	20.86 (15.96 - 21.53)			control (n=5)	32.61 (26.39 - 35.38)	
let7b	leukemia (n=21)	0.71 (0 - 636.49)	0.034*	miR150	leukemia (n=21)	35.31 (0 - 11051.85)	0.659
	control (n=5)	16.69 (15.76 - 18.06)			control (n=5)	24.2 (20.52 - 30.57)	
miR7	leukemia (n=21)	38.91 (0 - 21203.28)	0.569	miR155	leukemia (n=21)	127.47 (0 - 46148.62)	0.157
	control (n=5)	24.19 (22.4 - 25.64)			control (n=5)	28.5 (25.96 - 33.71)	
miR10	leukemia (n=21)	4.4 (0 - 6374.07)	0.374	miR181	leukemia (n=21)	3134.46 (0 - 5071566.49)	0.001*
	control (n=5)	25.38 (22.85 - 26.14)			control (n=5)	22.89 (17.58 - 24.89)	
miR21	leukemia (n=21)	353.61 (0 - 288058.04)	0.003*	miR192	leukemia (n=21)	49.11 (0 - 34588.38)	0.057
	control (n=5)	17.65 (11.94 - 24.2)			control (n=5)	22.16 (18.53 - 29.93)	
miR222	leukemia (n=21)	100.99 (0 - 52064.05)	0.003*	miR200b	leukemia (n=21)	136.62 (0 - 81471.06)	0.224
	control (n=5)	22.26 (18.15 - 25.96)			control (n=5)	33.08 (31.95 - 44.9)	
miR23	leukemia (n=21)	1.14 (0 - 1325.21)	0.157	miR200c	leukemia (n=21)	85.87 (0 - 55261.99)	0.659
	control (n=5)	16.1 (15.78 - 18.1)			control (n=5)	34.25 (29.59 - 35.67)	
miR27a	leukemia (n=21)	13.87 (0 - 4918.49)	0.659	miR218	leukemia (n=21)	2.82 (0 - 614.81)	0.012*
	control (n=5)	20.93 (20.08 - 26.99)			control (n=5)	29.03 (25.35 - 31.21)	
miR25	leukemia (n=21)	13.95 (0 - 1353.05)	0.486	miR331	leukemia (n=20)	0 (0 – 68.04x107)	0.001*
	control (n=5)	22.53 (19.26 - 24.9)			control (n=5)	18.64 (16.3 - 19)	
miR30	leukemia (n=21)	229.13 (0 - 42524.3)	0.023*	miR372	leukemia (n=21)	0 (0 - 0.24)	0.0001*
	control (n=5)	25.05 (13.36 - 29.01)			control (n=5)	20.93 (19.36 - 25.53)	
miR31	leukemia (n=21)	0 (0 - 0.98)	0.001*	miR375	leukemia (n=21)	0.01 (0 - 13.78)	0.0001*
	control (n=3)	17.78 (15.52 - 18.45)			control (n=5)	17.67 (17.65 - 25.04)	
miR132	leukemia (n=21)	61.65 (0 - 9449.35)	0.659	miR422	leukemia (n=21)	0.24 (0 - 388.02)	0.003*
	control (n=5)	29.89 (23.33 - 34.9)			control (n=5)	19.67 (14.56 - 24.89)	
miR135b	leukemia (n=21)	17.9 (0 - 81924.08)	1	miR451	leukemia (n=21)	0.57 (0 - 3800.57)	0.003*
	control (n=5)	21.09 (16.97 - 26.6)			control (n=5)	7.53 (5.33 - 10.54)	
miR128-1	leukemia (n=21)	1.28 (0 – 21.89x108)	0.049*	miR520	leukemia (n=20)	1.21 (0 – 54.82x1012)	0.042*
	control (n=5)	31.55 (25.78 - 35.67)			control (n=5)	32.6 (29.66 - 33.36)	
miR145	leukemia (n=21)	972.8 (0 - 739402.3)	0.012*	miR548	leukemia (n=21)	4.18 (0 - 14915.24)	0.659
	control (n=5)	27.4 (25.49 - 33.27)			control (n=5)	34.45 (21.35 - 36.8)	

and miR-155 increased and the expression of miR-375 decreased in patients with ALL.

Fold change analyses of miRNAs isolated from 21 patients diagnosed with acute leukemia before chemotherapy and from 17 of these patients one month after the therapy was performed. Two-fold or more increase was accepted as significant. As one of the patients had bone marrow transplantation, 2 were lost to follow-up and 1 did not complete one month of the treatment, their control miRNA analyses could not be performed. Although the miRNA with the highest increase was miR-331, it could not be accepted as statistically significant because it could be isolated from only 5 patients. According to this, miR-375 was the miRNA expression of which increased 30-fold higher in patients with leukemia. Apart from these, the expressions of miR-1, miR-23, miR-146a, miR 192, and miR-548 in patients with leukemia increased after the therapy while the expressions of let-7b and miR-132 decreased. When the leukocyte counts and miRNA expressions of the patients with leukemia before and after the therapy were compared, a positive moderate correlation was only found between miR-128-1 and miR-331 and leukocyte counts (p= 0.026; r= 0.537 and p= 0.037; r= 0.9 respectively). In the relationship between the percentage of blast

cells in bone marrow at the time of diagnosis and changes in miRNA before and after the therapy in patients with leukemia, a statistically significant increase was found in the expressions of miR-21 and miR-27a (p= 0.001, r= 0.74 and p= 0.08, r= 0.636 respectively). A negative correlation was only found between the percentage of blast cells on peripheral smear at the time of diagnosis and the expression of miR-200c (r= 0.586, p= 0.035).

DISCUSSION

Molecular cytogenetic findings and chromosomal changes in leukemia patients are important in determining risk groups and prognosis. Previously, Karkucak et al.[16] found that while 9p22 deletion was the most common abnormality in high-risk patients, t(12;21) was the most common abnormality in low and standard-risk groups in which our results are consistent. We also found that t(12;21) was the most common abnormality in low risk and standard risk ALL patients, but we did not find any chromosomal abnormality in high risk ALL patients.

Recently beside cytogenetic and chromosomal evaluation, the relationship between microRNAs and childhood hematologic cancers was evaluated in several studies. In the study of Duyu et al.,[17] miRNA analyses of 43 patients with ALL (34 with B-cell and 9 with T-cell) and the control group with 15 subjects were performed before the therapy and in the 6th month of the therapy and it was revealed that the expressions of miR-128, miR-146a, miR-155, miR-181a, and miR-195 increased in patients with ALL while the expressions of miR-146a, miR-155, miR-181a, and miR-195 significantly decreased 6 months after the therapy.

Let-7b located on 22q13.21 is a miRNA that is considered to have a tumor suppressor effect. It was stated in the study of Miu et al.[18] that let-7b was expressed more in patients with AML compared to the patients with ALL and can be used in the distinction of ALL and AML. However, Shafik et al. did not have similar results.[19] In our study, a significant decrease in let-7b was found in patients. No significant change in the expression of let-7b was observed after the therapy. This can be associated with the duration of treatment.

MiR-21 was among the most significant miRNAs that were associated with cancer genetics and progression. As it is associated with the growth, invasion, angiogenesis and metastasis of tumor through affecting apoptotic and tumor suppressor genes, oncogenic miRNA is mentioned as "oncomiR".[20] It was revealed in the study of Labib et al. that miR-21 was significantly upregulated in children diagnosed with ALL. Increased expressions in patients with ALL were associated with low thrombocyte counts, metastasis of central nervous system and drug resistance.[21] In our study, the expression of miR-21 increased in patients with acute leukemia and a statistically significant increase was also found in the expressions of miR-21 and miR-27a when the relationship between the percentage of blast cells in bone marrow and the changes in miRNA before and after the therapy was evaluated. As far as we have found out, there is no finding related to the relationship between miR-27a expression and childhood leukemia in literature.

MiR-128-1 (miR-128a) is located on 2q21.3. It was revealed in many studies that miR-128, associated with the hypomethylation of CpG, played a role in the pathogenesis of leukemia. It was found in the study of Mi et al.18 that the expressions of miR-128a and miR-128b were more in patients with ALL than in patients with AML. In the same study, it was stated that the accuracy rate of difference (97%) in the expressions of miR-223, a myeloid gene, miR-128a and miR-128b, and let-7b could be used in the diagnostic distinction of ALL and AML. It was revealed in the study of Shafik et al. that the expression of miR-128 was more in pediatric patients with ALL than in the control group.[19] Although no relationship between the expression of miR-128 and prognosis was found in this study, a relationship between low expression of miR-128 and poor prognosis was found in the study of Nemes et al.[22] We additionally found a directly proportional relationship between the expressions of miR-331 and miR-128-1 and the leukocyte count at the time of diagnosis. This relationship has not been stated in literature before.

MiR-204 located on 9q21.12 is a miRNA that is revealed to be suppressing JAK2 with proliferation, angiogenesis, and anti-apoptosis effects.[23] Butrym et al. revealed in their studies on adult patients diag-

nosed with AML that downregulation of miR-204 was associated with a decrease in the survival rate and that the increased expression of miR-204 after induction therapy was associated with remission.[24] It was also found in our study that the expression of miR-204 increased in patients with leukemia.

MiR-222 located on p11.3 with mi221 was revealed to inhibit proliferation in leukemic cells by affecting ETS1, a proto-oncogene in in vitro studies and induce cell cycle and apoptosis.[25] It was stated that miR-222 inhibited p27 protein expression with miR-221 and affected proliferation and CNS relapse in acute leukemia. Rommer et al.[26] found that the expressions of pri-miR-221 and 222 increased in patients with AML and were effective in leukemogenesis. Wang et al. found in their studies that miR-222 was overexpressed in patients with AML compared to patients with ALL.23 In our study, increased expression of miR-222 was found in patients with childhood leukemia.

MiR-331 located on 12q22 is a miRNA that takes place in the pathogenesis of cancer through activating STAT over the suppressor of cytokine signalling-1 and dysregulating JAK/STAT pathway.[27] In a study on cell culture consisting of acute leukemia cells, a negative correlation between doxorubicin resistance and P-glycoprotein increase important for its development and miR-331-5p and miR-27a was found. It was also stated that these microRNAs may be associated with relapse in the patients.[28] In our study, although the expression of miR-331 was low in patients with leukemia, there was a directly proportional relationship between the expression of miR-331 and leukocyte count at the time of diagnosis. In that case, the relationship between the change in the expression of miR-331 and the leukocyte count reveals its prognostic effect.

MiR375 located on 2q.35 is a microRNA that has been revealed to have tumor suppressor and oncogenic effect in different cancers by inhibiting proliferation, migration and invasion and promoting apoptosis by G1 cell cycle arrest.[29] Although Wang et al.[29] found that the expression of miR-375 increased in pediatric patients with AML compared to the control group, Bi et al. found in another study that miR-375 was downregulated especially in AML cells, which was associated with a poor prognosis in patients with AML. It was revealed that this effect of miR-375 occurred via miR-375-HOXB3-CDCA3/DNMT3B regulatory pathway and that both this miR-375 and this pathway could be used in the treatment of AML.[30] It was stated that the difference between these two studies may be due to the heterogeneity of the patient groups and cut-off levels of the defined expression of miR-375. In our study, in parallel with the findings of Bi et al., decreased expression of miR-375 was found in patients with AML and ALL. Increased expression of miR-375 was observed after the therapy.

In our study, we found a significant increase in the expression of miR-520e, a tumor suppressor miRNA belonging to the miR-520 family, in patients with leukemia. It was found in studies on miR-520 family that they had anti-oncogenic effect over TGF-B and NK-KB signal pathway in breast cancer and that they similarly had cancer suppressor effect in glioma cells.[31,32] In our study, increased expression was found in patients with leukemia. Increased expression of miR-520 was revealed for the first time in our study.

There is low number of studies on miR-548. In a study on the tissues of the patients with breast cancer, decreased expression of miR-548-3p was revealed.[33] In our study, although miR-548 expression did not differ in patients with acute leukemia, its increase after the therapy is a new finding.

While the strength of our study is that we analyzed several miRNAs in literature and performed prospective design, a low number of subgroups in patients with leukemia and non-controlled miRNA expressions when the therapy was completed may be the limitations of our study.

In conclusion, consistent with the literature, the relationship between leukocyte count at the time of diagnosis and the expressions of miR-331 and miR-128-1, the relationship between the percentage of blast cells and change in miR-27a, increased expression of miR-520 in patients with leukemia and increased expression of miR-548 after the therapy are new findings. To explain the effects of these miRNAs on childhood leukemia more clearly, further prospective studies with higher number of patients should be performed.

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