ÖZGÜN ARAŞTIRMA ORIGINAL RESEARCH

Med J SDU / SDÜ Tıp Fak Derg ▶ 2022:29(3):494-503 doi: 10.17343/sdutfd.1164324

THE POSSIBLE ROLE OF MIR-1910-3P, MIR-4649-3P, MIR-4296, AND MIR-210 IN THE PATHOGENESIS OF ATOPIC DERMATITIS: MAY MIR-4296 PLAY CRUCIAL ROLES IN THE DEVELOPMENT OF ATOPIC DERMATITIS?

ATOPİK DERMATİT PATOGENEZİNDE MİR-1910-3P, MİR-4649-3P, MİR-4296 VE MİR-210'UN OLASI ROLÜ: MİR-4296 ATOPİK DERMATİT GELİŞİMİNDE ÖNEMLİ ROL OYNAYABİLİR Mİ?

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Cite this article as: Ayvaz Çelik HH, Hekimler Öztürk K, Atay E, Erturan İ, Korkmaz S, Yıldırım M. The Possible Role of Mir-1910-3p, Mir-4649-3p, Mir-4296, and Mir-210 in The Pathogenesis of Atopic Dermatitis: May Mir-4296 Play Crucial Roles in The Development of Atopic Dermatitis? Med J SDU 2022; 29(3): 494-503.

Öz

Amaç
Atopik dermatit (AD), patogenezinde açıklanamayan noktaları olan kronik inflamatuar bir deri hastalığıdır. Plazmadaki mikroRNA'ların (miRNA, miR) değişmiş ekspresyonları, hastalıklı bireyleri sağlıklı kontrollerden ayıran belirteçler olarak hizmet edebilir. Bu çalışmada, AD'li hastalarda miR-1910-3p, miR-4649-3p, miR-4296 ve miR-210'un plazma ekspresyon düzeyleri araştırıldı.

Gereç ve Yöntem
Bu prospektif çalışmaya 40 AD'li hasta ve 40 sağlıklı kontrol alındı. MiRNA'ları ölçmek için real-time PCR kullanıldı.

Bulgular
Ortalama plazma miR-4296 düzeyi hasta grupta daha yüksek bulundu ($p < 0.001$). SCORAD skorları ile miR-210 seviyeleri arasında anlamlı bir negatif korelasyon saptandı (r:-0.340, p=0.032). miR-210 seviyeleri hastalık şiddeti arttıkça azalmaktaydı. Lojistik regresyon analizinde plazma miR-4296 seviyelerinde bir artış istatistiksel olarak anlamlı bulundu (OR =5.464, $p<0.001$). Diğer analizlerde anlamlı bir farklılık bulunmasa da miR-1910-3p seviyelerinde bir azalma da istatistiksel olarak anlamlıydı.

Sonuç
MiRNA'lar AD patogenezinde önemli rol oynamaktadırlar. Artan miR-4296 ekspresyon seviyeleri, AD hastalarını sağlıklı kişilerden ayırt etmede önemli ölçüde daha iyi görünmektedir.

Anahtar Kelimeler: Atopik dermatit, miR-4296, miR-1910-3p, miR-210, SCORAD

Abstract

Objective
Atopic dermatitis (AD) is a chronic inflammatory skin disease with unexplained points in its pathogenesis. Altered expressions of microRNAs (miRNA, miR) in

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Müracaat tarihi/Application Date: 19.08.2022 • Kabul tarihi/Accepted Date: 02.09.2022
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plasma can serve as markers that distinguish diseased individuals from healthy controls AD. In the present study, plasma expression levels of miR-1910-3p, miR-4649-3p, miR-4296 and miR-210 were investigated in AD.

Material and Method
Forty patients with AD and forty healthy control subjects were included in the present study. Quantitative real-time PCR was used to measure miRNAs.

Results
The mean plasma miR-4296 level was higher in the patient group ($p < 0.001$). There was a significant negative correlation between SCORAD scores and miR-210 levels (r:-0.340, p=0.032). miR-210 levels decreased with increasing disease severity. In logistic regression analyses, an increase in plasma miR-4296 levels was found to be statistically significant (OR =5.464, p<0.001). Moreover, although not significant in other analyzes, a decrease in the miR-1910-3p was shown statistically significant.

Conclusion
MiRNAs are crucial in the pathogenesis of AD. Increased miR-4296 seems to be significantly better at discriminating AD patients from healthy individuals.

Keywords: Atopic dermatitis, miR-4296, miR-1910-3p, miR-210, SCORAD

Introduction

Atopic dermatitis (AD) is an inflammatory skin disease that has significant negative impacts on the lives of patients and their families [1-3]. The enlightened part of the pathogenesis of AD includes barrier dysfunction, genetic factors, dysfunction in cell-mediated immunity, Immunoglobulin E (IgE)-mediated hypersensitivity and the imbalance of T lymphocytes, dysfunction in the synthesis of cytokines, antimicrobial peptides, chemokine, IgE, proteases, and their inhibitors involved in the correct structuring of epithelial cells, and environmental factors [2, 4, 5]. MicroRNAs (miRNAs, miRs) are silent non-coding RNAs that regulate the target genes' expressions post-transcriptionally [6]. Many studies reported that miRNAs could be detected in serum, urine, and amniotic fluid, and altered miRNA expression regulates many processes including progression, differentiation, maturation of cells, programmed cell death, and immune system homeostasis [7, 8]. Furthermore, many studies reported that miRNAs in plasma might serve as reliable markers to distinguish sick individuals from healthy controls, and determine the disease's severity and prognosis [9-13]. The miRNA topic is promising and popular in pathogenesis, genetics, and disease severity markers. They may become the regulators of future gene therapies in many dermatological diseases, also in AD [7]. Many authors evaluated many miRNAs in patients with AD [5, 6, 13, 14]. To our knowledge, no study is present evaluating plasma miR-1910-3p, miR-4649-3p, miR-4296, and miR-210 expression levels in AD. Few studies have been conducted on miR-210 alone in other allergic conditions such as asthma [15-17]. Thus, the current study was aimed to evaluate these miRs mentioned above, which target the Treg cells, and compare the results between patients with AD and healthy controls, and also to determine the potential of these miRs in the molecular pathogenesis of AD.

Material and Method

Ethics Committee of Suleyman Demirel University Faculty of Medicine approved this study (decision 10 on 16.01.2020) which was conducted in line with the principles of the "Helsinki Declaration". A signed consent form was obtained from all participants.

Study population: Patients diagnosed with AD according to Hanifin-Rajka criteria [18], with an age between 18-65, admitting to the dermatology clinic of our Hospital between February 2020- January 2021, were included in this study. Patients who has cardiovascular disease, active infection, and systemic diseases such as diabetes mellitus, rheumatic diseases, and/or inflammatory bowel diseases or other skin diseases than AD were excluded. All patients were selected from those who had not received any systemic therapy including phototherapy for at least 12 weeks before the study. The severity of the disease was evaluated with the SCORing Atopic Dermatitis (SCORAD) index [19] in the patient group and they were divided into three subgroups according to their SCORAD scores: mild disease with 0-24 score, moderate disease with 25-50 score, severe disease with >50 score.

Age- and sex-matched healthy volunteers with same criteria mentioned above were also enrolled as the control group.

miRNA extraction and cDNA synthesis: 4 ccs of peripheral venous blood from participants were

centrifuged, and the plasma portion was put into a 1.5 ml Eppendorf tube. The plasma was separated into Eppendorf tubes and stored at –80 ° C until miRNA isolation.

The isolation of miRNA from the samples was performed with the Hybrid-RTM miRNA Isolation Kit (GeneAll Biotechnology, Korea) according to instructions. The concentration and purity of the isolated total miRNAs were measured with a Thermo Fisher NanoDropTM spectrophotometer. The WScript ™ cDNA Synthesis Kit was used to obtain cDNA from the miRNA. Reverse transcription was performed using the SimpliAmp Thermal Cycler (Thermo Fisher Scientific, USA) according to instructions. The obtained cDNA samples were stored at -80ºC until quantitative real-time-PCR (qRT-PCR) analysis was performed.

Primer sequences and quantitation of candidate miRNAs: For miR-210: Forward: 5'-TAGCACCATTT-GAAATCGGTTA-3´(Accession No: MIMAT0000681). For miR-1910: Forward: 5'-AGGCAAGATGCTGG-CATAGCT-3'(Accession No: MIMAT0000089). For miR-4649: Forward: 5'-AGGCAAGATGCTGGCATAG-CT-3' (Accession No: MIMAT0000089). For miR-4296: 5'-TAAGGTGCATCTAGTGCAGATAG-3'(Accession No: MIMAT0000072), U6 snRNA as a housekeeping gene includes primers as follows: Forward: 5'-GCTT-CGGCAGCACATATACTAAAAT -3´.

For RNA integrity; on a gel safe dye stained %1.4 agarose gel, RNA integrity was tested by the presence of intact bands of 18S and 28S. The quantitation of the obtained miRNAs was carried out with Rotor-Gene Q (Qiagen, Hiden, Germany) following the manufacturer's instructions. miRNAs were analyzed using the SYBR green (miRCURY LNA SYBR Green PCR Kit cat no: 339346) method using U6 snRNA as an internal control (housekeeping gene). The cycle threshold (CT) values of miRNAs were determined and the obtained CT values were normalized to RNU6. The fold change of each miRNA expression was calculated using the 2 $2^{-\Delta\Delta Ct}$ equation.

Statistical Analysis

SPSS Statistics 27.0 was used to analyze data. The values of descriptives were presented as mean ± standard deviation. The categorical variables were presented as frequency and percentage, and Chi-Square analysis was used for them. The distribution characteristics were examined with the Shapiro Wilk test. Mann Whitney U or Kruskal Wallis test was used for non-parametric distributions according to the number of groups. For analysis of 2 scale/continuous type variables, Spearman correlation analysis was used. In logistic regression analysis, there are several methods for determining the independent variables that are added to the regression model. One of these methods is to select variables that are significant in univariate analyses. Although this is a common method, we preferred a different variant when building the model. Measurement of the expression level of miRs is relatively laborious and expensive. At this point, the evaluation of these parameters must be multidimensional. Therefore, we decided to include every possible related variable in the logistic regression model, even if significant or not. This is because in univariate analyses, some variables may not be significant due to masking of unpredictable conditions, and regression analyses can clarify this situation. In our analyses, a variable is observed in I 1910 that is not significant in the univariate analysis but is significant in the multivariate logistic regression. In all analyses, a p-value of <0.05 was accepted as significant.

Results

As shown in Table 1, 40 patients with AD and 40 controls were included. 12 (30%) of the patients were male and 28 (70%) were female, which is the same as the control group (p=0.999). The mean age of the patient group was 37.35 years, whereas it was 38.20 in the control group (p= 0.923). Descriptive features of the subjects are shown in Table 1. Of these patients, 11 (27.5%) had mild, 16 (40%) had moderate and, 13 (32.5%) had severe disease according to the SCORAD. Furthermore, 24 (60%) patients had a high level of serum IgE (>200 IU/mL). 4 (10%) patients had concurrent allergic asthma, 4 (10%) had allergic rhinitis, 3 (7.5%) had urticaria and, 1 (2.5%) had allergic conjunctivitis. In contrast, there have been no personal concurrent allergic diseases in the control group (p=0.001). 12 (30%) patients had an atopy history in their family, whereas 11 (27.5%) healthy controls had an atopy history in their family as well (p=0.999). These AD patients with other allergic diseases were not using regular medical treatment, and they had not used any systemic drug for at least 12 weeks, either. The mean levels of miR-1910-3p, miR-4649-3p, miR-4296 and miR-210 were compared between the two groups (Table 2). The mean miR-4296 level of the patient group was significantly higher (p<0.001) (Figure 1). While the levels of miR-1910-3p, miR-4649-3p, and miR-210 were compared, there was no significant difference (Table 2). In logistic regression analyses, an increase in plasma miR-4296 levels was seen statistically significant in developing AD (OR =5.464, p<0.001) (Table 3). Although there was no significant difference between the patient

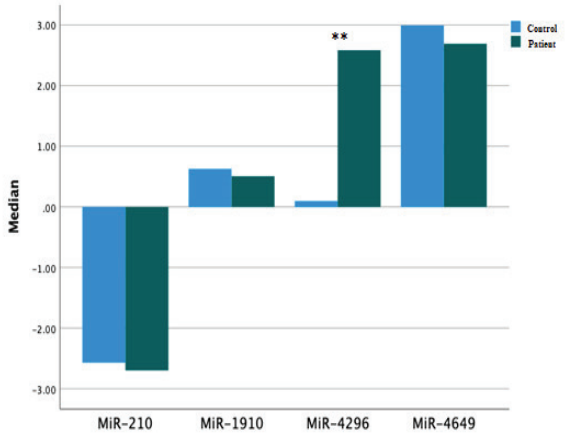

Figure 1:
Relative miRNA expression of AD according to healthy controls. **p<0.001, statistically significant. The relative expression value of the control group was accepted as 1. Expression levels of specific miRNAs: miR-210, miR-1910-3p, miR-4296, miR-4649-3p (FC=0.8, p=0.722; FC=1.4, p=0.312; FC=0.2, p=0.000, FC=0.9, p=0.501, respectively) in 40 AD patients, and 40 control were analyzed using parametric Mann-Whitney U Test. Data are presented as a median of normalized miRNA expression in log2 ($2^{-\Delta\Delta CT}$).

Table 1 Baseline characteristics of the study groups

	Patient group (n=40)			Control group (n=40)	p value*
Age, years	37.35 ± 14.29			38.20 ± 15.78	0.923
Female, n (%)	28 (70%)			28 (70%)	0.999
Male, n (%)	12 (30%)			12 (30%)	
	Mean ±Std†	Median	Minimum-Maximum		
Total IgE (IU/mL)	528.87 ± 624.19	529.37	10-2500		
Duration of disease, years	9.78 ± 9.2	10.16	1-35		
Mild disease, n (%) Moderate disease, n (%) Severe disease, n (%)	11 (27.5%) 16 (40%) 13 (32.5%)				
Other allergic conditions ‡	12 (30%)			0 (0%)	
Smokers, n (%) Non-smokers, n (%)	5 (12.5%) 35 (87.5%)			3 (7.5%) 37 (92.5%)	0.453
Alcohol users, n (%) Non-alcoholics, n (%)	9 (22.5%) 31 (77.5%)			2 (5%) 38 (95%)	0.712
Atopy in the family, n (%) No family history of atopy, n (%)	12 (30%) 28 (70%)			11 (27.5%) 29 (72.5%)	0.999

p*: Mann Whitney U †: Standart deviation, ‡: Concurrent allergic rhinitis, allergic conjunctivitis, asthma, urticaria

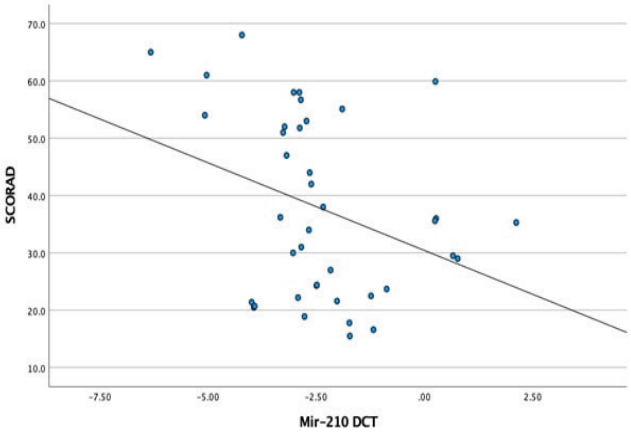

Figure 2:
Correlation graphic between miR-210 levels and SCORAD

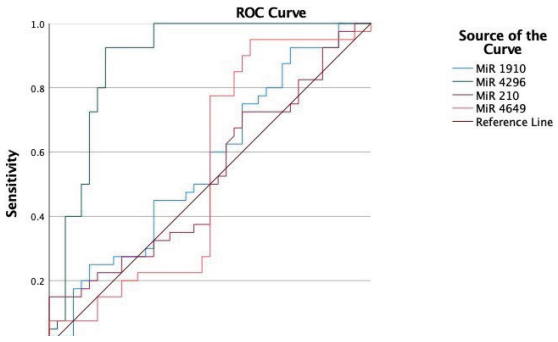

Figure 3:
Evaluation of plasma miRNA levels by ROC Curve Analysis

and the healthy control group, it was shown in the logistic regression analysis that the decrease in miR-1910-3p may be significant in the development of the disease (OR=0.421, p = 0.009) (Table 3). A significant negative correlation was observed between SCORAD and miR-210 levels (r:-0.340, p=0.032) (Table 4). According to this correlation analysis, miR-210 levels decreased as the disease got more severe (Figure 2). No correlation was seen between the severity of the disease and plasma miR-1910-3p, miR-4649-3p, and miR-210 levels (Table 4). Furthermore, no correlation was obtained between miRNA levels and groups according to serum IgE levels (Table 5). Moreover, the Receiver Operating Characteristic Curve (ROC) analysis revealed that AD patients could be significantly differentiated from healthy controls by plasma miR-4296 levels (Table 6, Figure 3).

Table 2 Baseline characteristics of the study groups

	N	Mean ±Std†	Median	Minimum	Maximum	p value*
miR-210						
Patients	40	-2.38 ±1.69	-2.69	-6.32	2.12	0.722
Controls	40	-2.69 ±1.55	-2.57	-6.78	-0.05	
miR-1910-3p						
Patients	40	0.51 ±1.28	0.50	-1.48	3.74	0.312
Controls	40	0.94 ±1.59	0.62	-1.37	4.47	
miR-4296						
Patients	40	2.68 ±0.93	2.58	1.02	5.57	**<0.001**
Controls	40	0.58 ±1.39	0.097	-1.09	4.43	
miR-4649-3p						
Patients	40	3.11 ±1.34	2.69	-0.03	6.40	0.501
Controls	40	3.04 ±1.59	2.99	0.31	5.65	

†: Standart deviation *p: Mann-Whitney U test

Table 3 Evaluation of miRNA levels and other parameters by logistic regression analysis

	B	Odds Ratio Exp(B)	95% CI for EXP(B)		P*
			Lower	Upper	
miR-210	.113	1.120	.620	2.022	0.708
miR-1910-3p	-.864	.421	.221	.803	**0.009**
miR-4296	1.698	5.464	2.680	11.140	**<0.001**
miR-4649-3p	.102	1.107	.683	1.794	0.679
Age	.002	1.002	.954	1.053	0.930
Sex	1.364	3.911	.545	28.071	0.175
Smoking	.203	1.225	.222	6.762	0.816
Alcohol intake	-.147	.864	.077	9.663	0.905
Constant	-2.294	.101			0.112

p*: Logistic regression analysis

Table 4 Correlations between plasma miRNA levels and SCORAD

	SCORAD	p value*
miR-210	-.340	**0.032**
miR-1910-3p	-.304	0.057
miR-4296	.036	0.824
miR-4649-3p	-.096	0.554

p*. Spearman correlation analysis (Non-parametric)

Table 5 Correlations between plasma miRNA levels and groups according to IgE levels

	N	Mean ±Std†	Median	Minimum	Maximum	p *
miR-210						
Normal IgE‡	16	-2.41±1.34	-2.68	-3.99	0.77	0.999
Higher IgE	24	-2.36±1.92	-2.72	-6.32	2.12	
miR-1910-3p						
Normal IgE‡	16	0.28 ±0.86	0.50	-1.16	1.61	0.692
Higher IgE	24	0.66 ±1.50	0.42	-1.48	3.74	
miR-4296						
Normal IgE‡	16	2.86 ±0.97	3.02	1.02	4.50	0.192
Higher IgE	24	2.55 ±0.89	2.49	1.24	5.57	
miR-4649-3p						
Normal IgE‡	16	3.05±1.36	2.62	0.86	6.40	0.754
Higher IgE	24	3.15 ±1.35	2.72	-0.03	5.88	

*p: Mann-Whitney U test †: Standart deviation ‡: Normal IgE level is between 0-200 IU/mL

Table 6 Evaluation of plasma miRNA levels by Roc curve analysis

	AUC*	p value**
miR-1910-3p	0.566	0.312
miR-4296	0.887	**<0.001**
miR-210	0.523	0.725
miR-4649-3p	0.544	0.501

* Area under the curve (AUC) P**<0.05

Discussion

In this study, we showed that increased miR-4296 expression level is particularly better at discriminating AD patients from healthy subjects 5.4 times. miR-4296 targets the mRNA of FOXP3 and inhibits it according to MicroRNA Target Prediction Database (miRDB). The expression of miR-4296 was higher in the patient group as expected. However, to our knowledge, there is no study about miR-4296 in AD till now. Previous studies reported an altered expression of miRNAs in the lesional skin or serum of patients with AD [20-28]. In these studies, increased expressions of let-7i, miR-24, miR-27a, miR-222, miR-21, miR-146a, miR-29a, miR-193a, miR-199a, miR-20a, miR17-5p, miR-106b and, decreased expressions of miR-326, miR-215, miR-122a, miR-133a, miR-133b were shown in the lesional skin of AD patients as well as in psoriasis [21, 22]. Furthermore, reduced expressions of miR-515-5p, miR-33, miR-483, and miR-519d have been found only in patients with AD [23].

In a recent study by Nousbeck et al, miRNA expression profiles were investigated in plasma samples of pediatric patients with AD. They showed eight dysregulated plasma miRNAs and ten differentially expressed miRNAs in peripheral mononuclear cells of AD infants compared to controls. They noticed that only the differential expression of miR-451a was similar in both plasma and mononuclear cells of the patients. They claimed that miR-451a could be a potential diagnostic marker for pediatric AD patients [24]. In our study, miR-4296 may serve as a discrimination marker for adult AD patients as well.

In a study by Lv et al, [25] a genome-wide miRNAs profiling was performed in pediatric patients with AD to determine any potential biomarkers. They showed that miR-203 and miR-483-5p levels were significantly elevated in the serum whereas paradoxically miR-203 was significantly decreased in the urine samples of the patient group. They also found an association between increased miR-203 in serum and the expression of sTNFRI and sTNFRII in pediatric patients with AD. In the same study, it was reported that patients with higher IgE levels had significant higher miR-203 expressions ($p=0.0011$). Furthermore, patients with normal or elevated serum IgE levels had significantly higher serum levels of miR-483-5p compared to healthy subjects ($p=0.0157$, $p=0.0094$, respectively). We did not determine any difference or correlation between expressions of miRNAs among patients according to serum IgE levels. Previous studies found significantly increased and decreased serum miR-125b and miR-146a levels in patients with AD [26, 27].

Dissanayake et al. tried to identify alteration of miRNAs between maternal serum and umbilical cord serum of pediatric AD patients. They showed elevated umbilical cord serum miR-144-3p level in the pediatric AD patients who were diagnosed in the first year of their lives [28]. Gu et al showed up-regulated miR-29b expressions in both lesional skin and serum of patients with AD than controls. Furthermore, they also showed that the serum level of miR-29b was positively correlated with the severity of the disease [29]. In our study, we established a negative correlation between miR-210 and SCORAD scores. Herberth et al. demonstrated increased miR-223 levels in maternal and cord blood, which was also correlated with lower Treg cell numbers. Therefore, they revealed that prenatal maternal tobacco smoke might result in increased expression of miR-223 level and increase the risk of development of AD in childhood [30].

In another study by our team, we compared serum expression levels of the same miRNAs as miR-4649-3p, miR-4296, miR-210, miR-1910-3p, and also miR-6867-5p between the patients with psoriasis and controls. We reported that serum miR-1910-3p levels were significantly lower and miR-4649-3p levels were significantly higher in the patient group. Furthermore, the decreased expression levels of miR-1910-3p could differ psoriatic patients from healthy controls [31]. In

the present study, Although there was no significant difference between the patient and the healthy control group, it was shown in the logistic regression analysis that the decrease in miR-1910-3p may be significant in the development of the disease. Based on this result, miR-1910-3p may have a role in the pathogenesis of AD, however, it needs to be investigated in studies with a larger sample.

In the present study, we also found that expression levels of miR-210 may alter according to the severity of AD disease. miR-210 is one of the most evaluated miRNAs in dermatologic and allergic diseases [15-17, 36-38]. In a study, miR-210 was found to increase in both psoriasis patients and mice models. They alleged that miR-210 promotes Th17 and Th1 cell differentiation, whereas it suppresses Th2 differentiation [36]. Likewise, in another study topical inhibition of miR-210 was shown to inhibit the psoriasis-like inflammation in the skin of mice [37]. Moreover, in a study miRNA expression profile of lesional skin samples of tumor stage mycosis fungoides (MF) was compared to skin samples of healthy controls. They found differently expressed 154 miRNAs between the two groups, and miR-29a, let-7a, miR-34a, and miR-210 were over-expressed in the patient group. Thus, they stated that these miRNAs may play significant roles in the pathogenesis of tumor stage MF disease [35]. In the present study, miR-210 levels were higher in patients, but this elevation was not significant. In addition, we also showed that the miR-210 level was negatively correlated with the severity of the disease. In a study by Long et al., the roles of miRNAs in chemical sensitization by toluene diisocyanate of occupational asthma were evaluated, and the expression level of miR-210 in lymph nodes of mice was reported. According to the results, the authors claimed that miR-210 might have an inhibitory role in the Treg function [15]. In another study, miR-210 was significantly correlated with airway obstruction in pediatric patients and, it was claimed that miR-210 might be necessary for the development of Th2 response in asthma [16]. A cell-culture study evaluating driving signalings for the differentiation of airway epithelium cells into smooth muscle especially in asthma patients showed miR-210 might have crucial roles in this process besides epidermal growth factor receptor [17]. In the present study, finding a negative correlation between miR-210 and SCORAD scores may show that miR-210 may help establish the severity of the disease in AD patients. Apart from AD and other dermatological conditions -especially malignancies-, many studies examine the expression of miRNAs that we evaluated in the present study. In these studies, authors claimed that miR-1910-3p may serve as a novel marker for the diagnosis of breast cancer [32], or may have protective roles for colorectal carcinoma [33]; miR-4649-3p serum levels have the potential to determine treatment responses in patients with malign melanoma [34]. In our study, there are some limitations, such as no inflammatory marker was studied apart from miRNAs. It is a cross-sectional study including a relatively small number of participants and the numbers of miRNA in this study were relatively low. Furthermore, participants in both groups who are smokers were included in this study. This fact may affect and alter the expressions of miRs [38]. Therefore, our results should be verified by prospective longitudinal future studies with a higher number of participants who are non-smokers, and the expressions of miR-1910-3p, miR-4649-3p, miR-4296, and miR-210 should be investigated in the lesional skin of patients with AD and mice models with AD.

This is the first report evaluating serum expression levels of miR-1910-3p, miR-4649-3p, miR-4296, and miR-210 in AD patients. We suggest that up-regulated miR-4296 in serum may be indicative of the development of disease in patients with AD, and miR-210 may serve as a marker to determine the disease severity in patients with AD. In the future, even detecting miRNAs in the perinatal period could show the development of diseases as well as AD in infancy. In addition, we also believe that miRNAs in serum and/or tissue may become the targets for future gene therapies in many diseases including dermatologic disorders in which cure therapies are very scarce.

Conflict of Interest Statement
The authors have no conflicts of interest to declare.

Ethical Approval
This prospective case-control study was approved by the Ethics Committee of Suleyman Demirel University, Faculty of Medicine (decision 10 on 16.01.2020) and was conducted in line with the principles of the "Helsinki Declaration".

Consent to Participate and Publish
Written informed consent to participate and publish was obtained from all individual participants included in the study.

Funding
This work was supported by the Suleyman Demirel University Scientific Research Projects Office (Project no TAB-2020-8244).
Availability of Data and Materials
Data are available on request due to privacy or other restrictions.

Authors Contributions

HHAÇ: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Resources; Validation; Visualization; Writing-original draft.

KHÖ: Conceptualization; Formal analysis; Investigation; Methodology; Project administration; Supervision; Validation; Writing-review & editing.

EA: Formal analysis; Investigation; Methodology; Validation; Writing-original draft.

İE: Investigation; Visualization; Writing-original draft.

SK: Supervision; Writing-review & editing.

MY: Supervision; Writing-review & editing.

Editorial

Although SK, one of the authors of the article, is editorial board member of the journal, she has not taken part in any stage of the publication processes of this article.

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