Araştırma Makalesi *Research Article*

Pamukkale Tıp Dergisi
Pamukkale Medical Journal

doi:https://dx.doi.org/10.31362/patd.1174388

Comparison of serum and lesional miRNA-1291 expressions in patients with bullous pemphigoid

Büllöz pemfigoidli hastalarda serum ve lezyonel miRNA-1291 ekspresyonlarının karşılaştırılması

Şule Subaşı Gökşin, Buket Er Urgancı, Işıl Göğem İmren, İbrahim Açıkbaş

Gönderilme tarihi:13.09.2022 Kabul tarihi:15.09.2022

Abstract

Purpose: Bullous pemphigoid (BP) is an autoimmune disease increasing by age. miRNA-1291 is a microRNA that plays major role in many immune-mediated cutaneous diseases. We aimed to investigate the role of miRNA-1291 in BP by comparing its expression levels in serum and lesional skin and its correlation with main BP biomarkers (anti-BP180, and anti-BP230) and CCL17.

Materials and methods: Twenty three patients with BP and 23 healthy control patients were included in the study. The miRNeasy Mini Kits was used for RNA isolation from serum and biopsy samples. Qiagen-miRCURY LNA RT Kit was used for conversion of cDNA. miRNA-1291 expression was performed by Real-Time PCR with the original primer and SYBR Green master mix. Serum CCL17, anti-BP180, and anti-BP230 levels were measured with use of ELISA. SPSS 25.0 (IBM SPSS Statistics 25 software) was used for statistical evaluation.

Results: The expression of miRNA-1291 in serum and biopsy samples were 7.02 and 4.77-fold significantly upregulated than that of control group respectively (p=0.001, p=0.007). The serum levels of anti-BP-180, anti-BP-230 and CCL17 in BP patients were significantly higher compared to control group (p=0.0049, p=0.0071, p=0.001). Anti-BP180, anti-BP230, CCL17 levels and serum miRNA-1291 expression were correlated positively in BP patients. Additionally, serum miRNA-1291 expression were correlated well with lesional miRNA-1291 expression.

Conclusion: The increase in miRNA-1291 levels in serum and lesional tissue compared to non-lesional tissue suggests that miRNA-1291 may play a role in the pathogenesis of BP. As miRNA-1291 correlates with other BP biomarkers, it may serve as an important biomarker for BP.

Key words: Bullous pemphigoid, biomarker, miRNA-1291, BP180, CCL17.

Subasi Goksin S, Er Urganci B, Gogem Imren I, Acikbas I. Comparison of serum and lesional miRNA-1291 expressions in patients with bullous pemphigoid. Pam Med J 2022;15:836-844.

Öz

Amaç: Büllöz pemfigoid (BP), yaşla birlikte artan otoimmün bir hastalıktır. miRNA-1291, birçok immün aracılı kutanöz hastalıkta önemli rol oynayan bir mikroRNA'dır. Serum ve lezyonlu derideki ekspresyon seviyelerini ve ana BP biyobelirteçleri (anti-BP180 ve anti-BP230) ve CCL17 ile korelasyonunu karşılaştırarak miRNA-1291'in BP'deki rolünü araştırmayı amaçladık.

Gereç ve yöntem: BP'li 23 hasta ve 23 sağlıklı kontrol hastası çalışmaya dahil edildi. Serum ve biyopsi örneklerinden RNA izolasyonu için miRNeasy Mini Kitleri kullanıldı. cDNA'nın dönüştürülmesi için Qiagen-miRCURY LNA RT Kiti kullanıldı. miRNA-1291 ekspresyonu, orijinal primer ve SYBR Green ana karışımı ile Real-Time PCR ile gerçekleştirildi. Serum CCL17, anti-BP180 ve anti-BP230 seviyeleri ELISA kullanılarak ölçüldü. İstatistiksel değerlendirme için SPSS 25.0 (IBM SPSS Statistics 25 yazılımı) kullanıldı.

Bulgular: Serum ve biyopsi örneklerinde miRNA-1291 ekspresyonu, kontrol grubuna göre sırasıyla 7.02 ve 4.77 kat anlamlı şekilde up-regüleydi (p=0,001, p=0.007). BP hastalarında antiBP-180, antiBP-230 ve CCL17 serum düzeyleri kontrol grubuna göre anlamlı derecede yüksekti (p=0.0049, p=0.0071, p=0.001).

BP hastalarında anti-BP180, anti-BP230, CCL17 seviyeleri, serum miRNA-1291 ekspresyonu ile pozitif korelasyon gösterdi. Ek olarak, serum miRNA-1291 ekspresyonu, lezyonel miRNA-1291 ekspresyonu ile iyi korele idi.

Sonuç: Serum ve lezyonlu dokuda lezyonlu olmayan dokuya göre miRNA-1291 düzeylerinin artması, miRNA-1291'in BP'nin patogenezinde rol oynayabileceğini düşündürmektedir. miRNA-1291 diğer BP biyobelirteçleri ile korelasyon gösterdiğinden, BP için önemli bir biyobelirteç olarak hizmet edebilir.

Anahtar kelimeler: Büllöz pemfigoid, biyobelirteç, miRNA-1291, BP180, CCL17.

Şule Subaşı Gökşin, Ass. Prof. Pamukkale University, Medical Faculty, Department of Dermatology, Denizli, Turkey, e-mail: sule.goksin@hotmail.com (https://orcid.org/0000-0001-8647-4298) (Corresponding Author)

Buket Er Urgancı, PhD. Pamukkale University, Medical Faculty, Department of Medical Genetics, Denizli, Turkey, e-mail: buketer@gmail.com (https://orcid.org/0000-0002-5339-3835)

Işıl Göğem İmren, M.D. Denizli State Hospital, Department of Dermatology, Denizli, Turkey, e-mail: isil.imren@gmail.com (https://orcid.org/0000-0002-9574-3231)

İbrahim Açıkbaş, Prof. Pamukkale University, Medical Faculty, Department of Medical Genetics, Denizli, Turkey, e-mail: iacikbas@pau.edu.tr (https://orcid.org/0000-0001-7483-1147)

Subaşı Gökşin Ş, Er Urgancı B, Göğem İmren I, Açıkbaş İ. Büllöz pemfigoidli hastalarda serum ve lezyonel miRNA-1291 ekspresyonlarının karşılaştırılması. Pam Tıp Derg 2022;15:836-844.

Introduction

Genetic predisposition, epigenetic changes and environmental factors are all responsible for the pathogenesis of autoimmune diseases. All these affect microRNAs (miRNAs) and trigger autoimmunity [1]. Recently discovered miRNAs have been shown to regulate and encode the expression of genes [2]. Cell growth, differentiation, apoptosis, immune response, regulation of cell adhesion molecules, cellular homeostasis are mainly regulated by miRNAs. Also, they are involved in translational suppression and/or degradation of target messenger RNA [3, 4].

The most commonly seen autoimmune bullous disease is bullous pemphigoid (BP). Autoantibodies against BP180 and BP230 proteins which are responsible for the adhesion of basal keratinocytes to the basement membrane region are produced by autoreactive B cells. This mechanism is mainly responsible for the pathogenesis of BP. Pathogenic autoantibodies target these two proteins, resulting in occurrence of tense bullae in the skin [5-8]. Anti-BP180 and 230 antibodies are biomarkers with a limited specificity and sensitivity for BP [9]. C-C chemokine ligand 17 (CCL17) is a well-recognized Th2 chemokine. Serum CCL17 levels have been reported to be significantly elevated in active BP patients [9].

BP is frequently seen in the elderly. Its morbidity and mortality are quite high [10]. Systemic or topical steroids as well as immunosuppressive drugs and intravenous immunoglobulin are used as treatment. However, all these treatments may result in serious complications in this type of patients [10]. Therefore, it is very important to discover new targets increasing success of treatment in order to reduce the morbidity and mortality of BP.

Increased expression of miRNA-1291, miRNA-27a-5p and miR-423-5p in serum of BP patients have been demonstrated in the literature. But, only miRNA-1291 reported to be significantly reduced after effective treatment. For this reason, miRNA-1291 could serve as an important serum biomarker for BP [10].

In our study, we targeted to investigate the presence of miRNA-1291 in serum and lesional tissue and its correlation with other known BP biomarkers such as anti-BP180, anti-BP230 and CCL17.

Materials and methods

Study population: Twenty-three patients with BP (13 females, 10 males) who applied to Pamukkale University Hospital Dermatology Clinic and 23 healthy volunteers (14 females, 9 males) matched by age and gender were included to the study.

Ethics and funding: Approval of the Ethics Committee of the Pamukkale University Non-Invasive Clinical Research was taken for this study. This study was supported by Pamukkale University Scientific Research Coordination Unit under the project number 2020BSP20.

Inclusion criteria: Patients diagnosed with BP clinically and histopathologically were included in our study.

Exclusion criteria: BP patients treated with systemic corticosteroids or immunosuppressants, not in the active stage of the disease, showing malignant tumors and/or other autoimmune diseases were excluded from the study.

Study design and setting: Prospective case-controlled study. Single centre tertiary hospital.

Collection of serum and tissue samples: A total of 5 mL of venous blood was drawn from the participants. Serum samples were isolated by 10 minutes of 2500 rpm centrifugation at 4°C, and stored at -80°C until used.

The biopsy samples from the lesional and nonlesional skin of the patients were collected with a 4-millimeter punch biopsy needle. Biopsy samples were placed in CA PBS (Phosphate Buffered Saline) (CA PBS-1A, DPBS (1x) without Ca & Mg, without Phenol Red- 500mL, Capricorn scientific, Westburg lifescience, Wesrburg, Holland), then lysed in Qiazol (Qiagen, Germany) and stored at -80°C until used.

Serum miRNA isolation: Serum MiRNeasy Mini Kit (Qiagen, Germany, Cat.No.217184) was used for RNA isolation from serum samples. A total of 200 µl of serum samples were taken, and 1000 µl of QIAzol Lysis Solution was added and mixed by pipetting. Tubes containing the lysate were incubated at room temperature (15-25°C) for 5 minutes. 200 µl of chloroform was added to the lysate and mixed by rapid vortexing for 15 seconds and left at room temperature for 2-3 minutes. Afterward, it was centrifuged at 12,000 x g, 4°C for 15 minutes. Three different interphases containing RNA have been obtained. The white interphase was transferred to a new tube. 1.5 times 100% ethanol was added and mixed by pipetting. Approximately 700 µl of the sample was transferred to the RNeasy MinElute spin column. Centrifugation was done at 8000 x g for 15 seconds at room temperature. The same step was repeated for the remaining sample. Afterward, 700 µl of RWT buffer was added to the spin column. Centrifugation was done at 8000 x g for 15 seconds at room temperature. 500 µl of RPE buffer was added to the spin column. Centrifugation was done at 8000 x g for 15 seconds at room temperature. The spin column was taken into a new 2 ml collection tube and centrifuged at the highest speed for 5 minutes to dry the membrane. The spin column was transferred into a new 1.5 mL collection tube, and 14 µl of RNase-free water was added to the center of the column membrane and centrifuged at the highest speed for 1 minute. Approximately 12 µl of total RNA was obtained.

Isolation of tissue miRNA: The miRNeasy Mini Kit (Qiagen, Germany, Cat.No.217004) was used for RNA isolation from biopsy samples. The samples in trizol were removed from the -80°C storage place and allowed to melt at room temperature. Melted biopsy samples were taken into a petri dish and homogenized with a scalpel by adding 700 µl of triazole. The homogenized samples were taken into 1.5 mL Eppendorf tubes and 140 µl of chloroform was added. After cortex was performed for 15 seconds, it was incubated for 3 minutes at room temperature. It was centrifuged at 12000 g at +4°C for 15 minutes. Afterward, the supernatant was transferred into a new 1.5 mL Eppendorf tube. 1.5 times as much as 96% ethanol (approximately 525 µl) was added to the supernatant transferred to the Eppendorf and pipetted slowly. 700 µl of the samples were placed on spin columns. It was centrifuged at 8000 g for 30 seconds at room temperature and the filtrate was removed. 700 µl of RWT buffer was added to the column and centrifuged at 8000 g for 15 seconds and the filtrate was removed. 500 µl of RPE buffer was added to the column and centrifuged at 8000 g for 15 seconds, then, the filtrate was removed. Afterward, 500 µl of RPE buffer was added to the spin column and centrifuged at 8000 g for 2 minutes. For the final drying phase, the spin column was transferred to a new 2 ml collection tube and centrifuged for 1 minute at the highest speed. Spin columns were placed in new 1.5 mL collection tubes and 30 µl of RNase-free water was placed in the middle of the spin column and centrifuged at 8000 g for 1 minute. Concentration and purity determinations of RNAs were performed with Nanodrop device. RNAs that were not converted to cDNA were removed to -80°C storage.

Synthesis of cDNA: For miRNA expression analysis, cDNA conversion was performed with miRCURY LNA RT Kit (Qiagen, Germany, Cat. No.339340). The reaction mixture was prepared with the amounts given in Table 1.

The reaction was carried out by incubating the tubes containing the prepared reaction mixture for 60 minutes at 37°C and then for 5 minutes at 95°C.

Expression analysis: miRNA-1291 miRCURY primer assay (Qiagen, Germany, Cat. No.339306) was used for expression analysis and expression profiles were calculated using the U6 miRCURY primer assay (Qiagen, Germany, Cat.No.339306) as the reference. Real-time PCR tests were performed by Rotor-Gene (Qiagen) according to the miRCURY LNA SYBR® Green PCR Kit (Qiagen, Germany, Cat. No.339345) protocol. The reaction mixture was prepared with the amounts given in Table 2.

miRNA-1291 reaction conditions;

95°C 15 minutes

94°C for 15 seconds,

58°C for 30 seconds,

72°C 30 seconds.

Expression changes were determined by the 2-ΔΔCT method.

Table 1. Reaction mixture prepared for cDNA synthesis

Substance	Amount
5x HiFlex Buffer	4 µl
10x MiScript Nucleics Mix,	2 µl
MiScript Reverse Transkriptaz Mix	2 µl
RNA	5 µl
dH2O	7 µl
Total	**20µl**

cDNA: Complementary DNA
dH2O: Distilled water

Table 2. Real-Time PCR reaction mixture

Substance	Amount
SYBR green PCR mastermix	5 µl
Forward Primer	0.5 µl
Reverse Primer	0.5 µl
cDNA	2 µl
dH_2O	2 µl
Total	**10 µl**

PCR: Polymerase Chain Reaction
SYBR: Safe stain specifically formulated to be a safer alternative to ethidium bromide
cDNA: Complementary DNA
dH2O: Distilled water

Determination of CCL17, anti-BP180, anti-BP230: CCL17, anti-BP-180, and anti-BP-230 levels were determined by ELISA testing of serum samples of patient and control groups. Cytokine determination was performed with Shanghai sunredbio (SRB) brand kit. In this system, based on the biotin double antibody (sandwich) principle, the wells are coated with antibodies specific to cytokines. Standards were prepared at different concentrations for each kit (Table 3).

Table 3. Standard concentrations

	CCL17	Anti-BP180	Anti-BP230
Standard 1	2400 ng/ml	12 U/L	16 U/L
Standard 2	1200 ng/ml	6 U/L	8 U/L
Standard 3	600 ng/ml	3 U/L	4 U/L
Standard 4	300 ng/ml	1.5 U/L	2 U/L
Standard 5	150 ng/ml	0.75 U/L	1 U/L

Anti-BP180: Bullous pemphigoid antigen II (BP180) autoantibody
Anti-BP230: Bullous pemphigoid antigen I (BP230) autoantibody
CCL17: C-C chemokine ligand 17

50 µl of standard and 50 µl of Streptavidin-HRP were added to the standard well. 40 µl of the sample, 10 µl of antibody, and 50 µl of Streptavidin-HRP were added to the sample wells. After 60 minutes of incubation at 37°C, washing was performed with the washing solution. First, 50 µl of chromogen solution A and 50 µl of chromogen solution B were added to the wells, respectively. After 10 minutes of incubation at 37°C in the dark, 50 µl of stop solution was added (blue color will turn yellow). The color change and absorbances were detected at 450 nm wavelength. The concentrations of the samples were calculated by comparison with the standards.

Statistical analysis

Data were analyzed with SPSS 25.0 (IBM SPSS Statistics 25 software) (Armonk, NY, IBM Corp. USA) package program. Continuous variables are expressed as mean ± standard deviation. The conformity of the data to the normal distribution was examined with the Shapiro-Wilk test. When parametric test assumptions were

not met, Kruskal Wallis analysis of variance (post hoc: Mann Whitney U test with Bonferroni correction) and Mann-Whitney U test were used to compare independent group differences. Spearman correlation analysis was used to analyze the relationships between numerical variables. RT-PCR data were analyzed with the 2-ΔΔCT method and quantitated with an online program (https://dataanalysis2.qiagen.com/pcr). The comparison of the groups has been performed with a "Volcano Plot" analysis, from "RT2-Profiles™PCR Array Data Analysis", which is assessed statistically using the "Student's t-test". *P*<0.05 was considered statistically significant.

Results

Demographic analysis: The mean age of the patients was 65.63±11.78 years, and this was 64.9±11.99 years in control group. There was no statistically significant difference between the groups in terms of age and gender (*p*=0.087).

miRNA-1291 expression levels in tissue and serum: The expression of miRNA-1291 in patient serum samples was 7.02 times higher in BP patients. 4.77-fold and 1.74-fold increases were observed in the lesional and non-lesional tissue of the patients, respectively. miRNA-1291 expressions in the non-lesional tissue of BP patients were similar to the control group. (Table 4).

Serum Anti-BP180, Anti-BP230, and CCL17 ELISA levels: Anti-BP180, anti-BP230 and CCL17 serum levels were statistically significantly higher in BP patients compared to the control group (*p*=0.0049, *p*=0.0071, *p*=0.001) (Table 5).

Correlation between anti-BP-180, anti-BP-230, CCL17 and miRNA-1291 levels: Anti-BP-180, anti-BP-230, CCL17 levels, and serum miRNA-1291 expression were correlated positively in BP patients. Serum miRNA-1291 and tissue miRNA-1291 expressions were also positively correlated (Table 6).

Table 4. miRNA-1291 expressions as a fold in the serum, lesional and nonlesional tissues of patients with BP compared to control group

	Serum	Lesional Tissue	Non-lesional Tissue
miRNA 1291	7.02	4.77	1.74
***p* value**	**0.001**	**0.007**	0.14

miRNA 1291: Mikro RNA

Table 5. Serum anti-BP-230, anti-BP180, and CCL17 levels in the BP patients and control group

	Group	Mean ±SD	*p* value
Anti-BP180	BP	105.6±39.9 U/ml	**0.0049**
	Control	22.1±15.6 U/ml	
Anti-BP230	BP	79.8±35.3 U/ml	**0.0071**
	Control	24.7±22.1 U/ml	
CCL17	BP	1253.96±844.27 pg/ml	**0.001**
	Control	252.36±180.76 pg/ml	

Anti-BP180: Bullous pemphigoid antigen II (BP180) autoantibody
Anti-BP230: Bullous pemphigoid antigen I (BP230) autoantibody
CCL17: C-C chemokine ligand 17

Table 6. Correlations between ELISA results and miRNA expressions

			Correlations				
			Anti-BP180	**Anti-BP230**	**CCL17**	**miRNA-1291 in serum**	**miRNA-1291 in tissue**
Spearman's rho	Anti-BP180	Correlation Coefficient	1.000	**.326***	**.348***	**.356***	.295
		Sig. (2-tailed)	.	**.03**	**.02**	**.021**	.058
		N	46	46	46	46	46
	Anti-BP230	Correlation Coefficient	**.326***	1.000	.291	**.340***	.281
		Sig. (2-tailed)	**.03**	.	.062	**.028**	.072
		N	46	46	46	46	46
	CCL17	Correlation Coefficient	**.348***	.291	1.000	**.412****	.302
		Sig. (2-tailed)	**.02**	.062	.	**.001**	.052
		N	46	46	46	46	46
	miRNA1291 in serum	Correlation Coefficient	**.356***	**.340***	**.412****	1.000	**.382***
		Sig. (2-tailed)	**.021**	**.028**	**.001**	.	**.010**
		N	46	46	46	46	46
	miRNA1291 in tissue	Correlation Coefficient	.295	.281	.302	**.382***	1.000
		Sig. (2-tailed)	.058	.072	.052	**.010**	.
		N	46	46	46	46	46

*. Correlation is significant at the 0.5 level (2-tailed)
**. Correlation is significant at the 0.01 level (2-tailed)
Anti-BP180: Bullous pemphigoid antigen II (BP180) autoantibody
Anti-BP230: Bullous pemphigoid antigen I (BP230) autoantibody
CCL17: C-C chemokine ligand 17
miRNA 1291: Mikro RNA 1291

Discussion

The incidence of BP increases markedly by age. Generally, it is prevalent in women older than 70-year-old [11]. The mean age of the patients in our study was 65, and more than half of them were female.

miRNAs are closely related to both the regulation of the normal development of the immune system and the pathogenesis of chronic inflammatory disorders [2]. Although the etiopathogenetic relationship among chronic spontaneous urticaria, psoriasis, and other chronic inflammatory dermatological diseases is not clearly understood, evidence about the act of miRNAs in these disease's pathogenesis is getting rapidly increased [12]. The miRNA-related control process of autoimmune reactions is highly sophisticated due to miRNA variety and the multitargeted character of these molecules [13].

In a recent study, it was reported that serum MiRNA-1291 was significantly increased in active BP patients, and its expression could reflect BP activity with a sensitivity of 75.56% and a specificity of 81.03% [10]. In this study, levels of serum miRNA-1291, miRNA-27a-5p, and miRNA-423-5p were found increased, only miRNA-1291 level was found reduced significantly after effective treatment in BP patients [10]. miRNA expression in lesional skin wasn't investigated in this study. However, in our study, we found a statistically significant increase in miRNA 1291 expression level in both lesional skin and serum. Moreover, we also found that there was a positive correlation between serum miRNA and lesional miRNA levels.

Potential BP-associated target genes for miRNA-1291 were STAT6 and IL-13. STAT6 expression was increased significantly in lesional tissue, not in perilesional skin and normal skin [14]. In BP, imbalance of regulatory T cells has been shown to result in Th2 cytokine production, STAT6 signaling pathway-induced activation of CD4+ autoreactive Th2 cells, and hence, synthesis of characteristic pathogenic autoantibodies [15]. In our study, target genes of miRNA-1291 weren't studied.

Also, we found a statistically significant positive correlation between miRNA-1291 and anti-BP180, 230 and CCL17. In the study of Qiu et al. [10], miRNA levels were reported to correlate with baseline levels of serum CCL17 and anti-BP180 antibodies.

CCL17 are considered important chemokines involved in the pathogenesis of BP [16]. Serum CCL17 levels are also associated with BP disease activity [17].

In another study with 20 BP patients, CCL17 levels were found to be correlated with the BP Disease Area Index (BPDAI) and urticaria/erythema scores [18-21]. Since BPDAI and erythema were not scored in our study, we could not determine whether there is a relationship between CCL17, anti-BP180, anti-BP230, miRNA levels and BP disease severity.

In this study, we also found a statistically significant correlation among serum miRNA-1291 levels and anti-BP180, 230 and CCL17 level.

To the best of our knowledge, this is the first study describing miRNA expression in the lesional skin of BP patients. Although there is no certain evidence of a clear relationship between miRNAs and cutaneous diseases, the data of our study may provide a promising basis for the diagnosis and treatment of BP.

As a result, the increase in miRNA-1291 levels in serum and lesional tissue compared to non-lesional tissue suggests that miRNA-1291 may play a role in the pathogenesis of BP. Since miRNA-1291 is associated with other BP biomarkers, miRNA-1291 may serve as an important biomarker for BP both at diagnosis and in evaluation of response to therapy.

Further studies determining the role of miRNA-1291 and target genes may provide new options in the BP treatment.

We did not evaluate erythema scores and disease severity (BP Disease Area Index [BPDAI]) in our study. Therefore, we were unable to determine whether miRNA was affected by these variables. miRNA-1291 levels were not evaluated after BP treatment. The increase of miRNA-1291 in both serum and lesional tissue indicates that miRNA-1291 may have a role in the etiopathogenesis of BP. Research on the in vivo functions of miRNA-1291 and the genes targeted by miRNA-1291 has not been conducted. New studies are needed to shed

light the target genes and pathways related to miRNA-1291.

Conflict of interest: No conflict of interest was declared by the authors.

References

1. Zhao CY, Murrell DF. Outcome measures for autoimmune blistering diseases. J Dermatol 2015;42:31-36. https://doi.org/10.1111/1346-8138.12711
2. Sonkoly E, Ståhle M, Pivarcsi A. MicroRNAs: novel regulators in skin inflammation. Clin Exp Dermatol 2008;33:312-315. https://doi.org/10.1111/j.1365-2230.2008.02804.x
3. Iorio MV, Croce CM. MicroRNA dysregulation in cancer: diagnostics, monitoring and therapeutics. A comprehensive review. EMBO Mol Med 2012;4:143-159. https://doi.org/10.1002/emmm.201100209
4. Chen JQ, Papp G, Szodoray P, Zeher M. The role of microRNAs in the pathogenesis of autoimmune diseases. Autoimmun Rev 2016;15:1171-1180. https://doi.org/10.1016/j.autrev.2016.09.003
5. Genovese G, Di Zenzo G, Cozzani E, Berti E, Cugno M, Marzano AV. New insights into the pathogenesis of bullous pemphigoid: 2019 Update. Front Immunol 2019;10:1506.e1-8. https://doi.org/10.3389/fimmu.2019.01506
6. Chiorean RM, Baican A, Mustafa MB, et al. Complement-activating capacity of autoantibodies correlates with disease activity in bullous pemphigoid patients. Front Immunol 2018;9:2687. https://doi.org/10.3389/fimmu.2018.02687
7. Amber KT, Murrell DF, Schmidt E, Joly P, Borradori L. Autoimmune subepidermal bullous diseases of the skin and mucosae: clinical features, diagnosis, and management. Clinic Rev Allergy Immunol 2018;54:26-51. https://doi.org/10.1007/s12016-017-8633-4
8. Liu Y, Li L, Xia Y. BP180 is critical in the autoimmunity of bullous pemphigoid. Front Immunol 2017;8:1752.e1-15. https://doi.org/10.3389/fimmu.2017.01752
9. Feliciani C, Caldarola G, Kneisel A, et al. IgG autoantibody reactivity against bullous pemphigoid (BP) 180 and BP230 in elderly patients with pruritic dermatoses. BJD 2009;161:306-312. https://doi.org/10.1111/j.1365-2133.2009.09266.x
10. Qiu L, Zhang L, Qi R, Gao X, Chen H, Xiao T. miR-1291 Functions as a potential serum biomarker for bullous pemphigoid. Dis Markers 2020;2020:9505312.e1-10. https://doi.org/10.1155/2020/9505312
11. Marazza G, Pham HC, Schärer L, et al. Autoimmune bullous disease Swiss study group. Incidence of bullous pemphigoid and pemphigus in Switzerland: a 2-year prospective study. BJD 2009;161:861-868. https://doi.org/10.1111/j.1365-2133.2009.09300.x
12. Bakay ÖSK. Kronik spontan ürtiker ile microrna-221, microrna-155 ve IL-31 ilişkisinin araştırılması - Investigation of relationship between chronic spontaneous urticaria with microrna-221, micorna-155 and IL-31. Erişim adresi: https://tez.yok.gov.tr/UlusalTezMerkezi/tezSorguSonucYeni.jsp. Erişim tarihi 20 Ağustos 2022
13. Zurawska A, Mycko MP, Selmaj KW. Circular RNAs as a novel layer of regulatory mechanism in multiple sclerosis. J Neuroimmunol 2019;334:576971.e1-8. https://doi.org/10.1016/j.jneuroim.2019.576971
14. Juczynska K, Wozniacka A, Waszczykowska E, et al. Expression of the JAK/STAT signaling pathway in bullous pemphigoid and dermatitis herpetiformis. Mediators Inflamm 2017;2017:6716419.e1-13. https://doi.org/10.1155/2017/6716419
15. Muramatsu K, Ujiie H, Kobayashi I, et al. Regulatory T-cell dysfunction induces autoantibodies to bullous pemphigoid antigens in mice and human subjects. J Allergy Clin Immunol 2018;142:1818-1830. https://doi.org/10.1016/j.jaci.2018.03.014
16. Vestergaard C, Deleuran M, Gesser B, Larsen CG. Thymus- and activation-regulated chemokine (*TARC/CCL17*) induces a Th_2-dominated inflammatory reaction on intradermal injection in mice. Exp Dermatol 2004;13:265-271. https://doi.org/10.1111/j.0906-6705.2004.00149.x
17. Tamaki K, Kakinuma T, Saeki H, et al. Serum levels of CCL17/TARC in various skin diseases. J Dermatol 2006;33:300-302. https://doi.org/10.1111/j.1346-8138.2006.00072.x
18. Kakinuma T, Wakugawa M, Nakamura K, Hino H, Matsushima K, Tamaki K. High level of thymus and activation-regulated chemokine in blister fluid and sera of patients with bullous pemphigoid. BJD 2003;148:203-210. https://doi.org/10.1046/j.1365-2133.2003.05066.x
19. Saeki H, Tamaki K. Thymus and activation regulated chemokine (TARC)/CCL17 and skin diseases. J Dermatol Sci 2006;43:75-84. https://doi.org/10.1016/j.jdermsci.2006.06.002
20. Echigo T, Hasegawa M, Shimada Y, Inaoki M, Takehara K, Sato S. Both Th_1 and Th_2 chemokines are elevated in sera of patients with autoimmune blistering diseases. Arch Dermatol Res 2006;298:38-45. https://doi.org/10.1007/s00403-006-0661-5
21. Suzuki M, Yamaguchi Y, Nakamura K, et al. Serum thymus and activation-regulated chemokine (TARC/CCL17) may be useful to predict the disease activity in patients with bullous pemphigoid. JEADV 2021;35:121-124. https://doi.org/10.1111/jdv.16851

Funding: This study was supported by Scientific Research Coordination Unit of Pamukkale University under the project number 2020BSP20.

Ethics committee approval: Permission was obtained from Pamukkale University Non-Invasive Clinical Research Ethics Committee for the study (date: 3.12.2021, number: 10.150.1.99/4822540).

Authors' contributions to the article

S.S.G. have constructed/constructed the main idea and hypothesis of the study. S.S.G. and B.E.U. developed the theory and arranged/edited the material and method section. S.S.G., B.E.U., I.G.I. and I.A. have done the evaluation of the data in the Results section. Discussion section of the article written by S.S.G., reviewed, corrected and approved by S.S.G. In addition, all authors discussed the entire study and approved the final version.