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MicroRNA548ac expression level in relation to BDCAF scored Behçet's disease activity and history of treatment response

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Abstract

Background: Behçet's disease gives a challenge to be diagnosed and followed up due to lack of specific biomarkers. MicroRNAs showed relations to different disease states including immunological and inflammatory illnesses. In this study, we are estimating microRNA548ac levels for the first time to be tested in the disease to see if there is a link to disease activity and if microRNA548ac can be used as a biomarker for activity or remission and prognosis of Behçet's disease. MicroRNA548ac has been shown to have a role in autoimmunity and some inflammatory conditions. Blood samples were taken from patients to measure white blood cells expression of microRNA548ac, and compared to its expression in healthy subjects, disease activity was assessed by usage of Behçet's Disease Current Activity Form (BDCAF).

Results: MicroRNA548ac expression decreased but not significantly with increased Behçet's disease activity, and expression was having a significant positive correlation with increased treatment response history.

Conclusions: MicroRNA548ac appeared not to be related to disease activity which needs confirmation in further studies, but it may predict response to treatment so that patients having higher expression of microRNA548ac may have a better response to treatment. Here, microRNA548ac could be used as a disease biomarker for disease prognosis.

Keywords: Behçet's disease, MicroRNA548ac, BDCAF

Background

Behçet's disease (BD) is one of the vasculitides, which is characterized by many systemic manifestations, including oral ulcers, genital ulcers, skin lesions, ocular diseases, neurologic manifestations, rheumatologic complaints (arthralgia ± arthritis), and gastrointestinal disease. These clinical manifestations of BD are due to blood vessel inflammation mainly. The pathogenesis of BD is partially understood, but roles of genetic and epigenetic factors in disease pathophysiology were demonstrated in some studies. It is observed that geographic distribution in BD may reflect genetic susceptibility or environmental factors in some populations. BD prevalence increases in the Silk Road countries, although it occurs world-wide. Reported prevalence rates are 2.1–420/100,000 inhabitants of Asia and North Africa populations, and 0.3–7.5/100,000 inhabitants of Western Europe or the USA [1–3].

MicroRNAs (miRNAs) are endogenous, short non-coding RNAs. They bind to target mRNAs, which leads to translational suppression or degradation of respective mRNAs. MiRNAs play roles in many physiological processes including differentiation, development, proliferation, and apoptosis. They appeared to be expression regulators of genes in acquired and innate immune system controlling antibody production and release of

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inflammatory mediators. Abnormal miRNAS expression and functioning is linked to many diseases like inflammatory disorders, cancers, infectious diseases, etc., where they are upregulated or downregulated. Hence, measuring or targeting their expression of might serve as a strategy for diagnosis, prevention, or treatment of various diseases [4].

MicroRNA548ac (miRNA548ac) has shown some evidence to be involved in immune response and pathogenesis of some inflammatory conditions [5, 6]; this makes it important to investigate its level in BD which is considered an autoimmune and auto-inflammatory disease [3]. Some miRNAs were shown to be expressed with good treatment response [7, 8].

Aim of the work

Aim of our work was to show miRNA548ac expression in disease activity of BD that is scored by BDCAF to know if it is possible to use it as a marker of BD activity or remission and if it could be used as a measure of treatment response and prognosis of the disease.

Methods

Recruitment of twenty-three Egyptian BD patients fulfilling the International Study Group diagnostic Criteria of BD and international criteria for BD were examined [9, 10]. Patients with another auto-inflammatory, another autoimmune disease, other vasculitis, and hyper-coagulable states were excluded; history and examination were done; lab investigations including HLAB51 and radiological investigations if were needed were done to asses patients; and blood samples were obtained after a verbal informed consent was taken from patients and Ethics Committee approved for that ad provided a permission for the study.

- Measurement of BD activity utilizing Behçet's disease activity form "BDCAF" [11] score depending on: The presence of oral ulcers, genital ulcers, arthritis, arthralgia, pustules, erythema nodosum, headaches, new involvement of central nervous system, new eye inflammation, new major vessel event, diarrhea, and nausea/vomiting over the 4 weeks before the clinical visit. Accordingly division of the patients into two groups was done: a higher disease activity group (nine patients; 39.135) with a score equals to or more than 4 out of 12, and a lower disease activity group (fourteen patients; 60.86%) with a score less than 4, Table 1.
- Treatment response: by comparing BDCAF activity score since the beginning of illness and the BDCAF when samples are taken mostly after treatment, patients' division into two groups according to treatment response calculated by subtraction of BDCAF score of the first presentation of the disease by recent BDCAF score divided by the former score to give a percentage. The two groups are a higher treatment response group (fourteen patients) with response to treatment more than 50% and a lower treatment response group (nine patients) with response to treatment less than 50%, taking in consideration that two patients were not on treatment at the time of taking samples.
- Control group: ten subjects matched to patients by age and sex.

Table 1 Classification of patients according to BDCAF score

BDCAF activity score	
0	2 (8.7%)
1	4 (17.4%)
2	3 (13%)
3	5 (21.7%)
4	7 (30.4%)
5	0 (0%)
6	1 (4.3%)
7	1 (4.3%)
Min.–Max.	0–7
Mean ± SD	2.9 ± 1.8
Median (IQR)	3 (1.5–4)

MiRNA548ac expression was measured by TaqMan® applied biosystems through successive steps. In the first white blood cells, the total RNA was purified and put in −80°C, next the reverse transcription (RT) reaction master mix followed by RT reaction were prepared, then reverse transcription and the qPCR amplification were performed; after that, the qPCR reaction mix and the PCR reaction plate were prepared, then the analysis was done [12–14].

Results

Statistical analysis of the data

Data were analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp). The Kolmogorov-Smirnov: to verify the normality of variable distribution. Chi-square test (Fisher's exact): Comparisons between groups (for categorical variables). Student *t* test: to compare two groups (for quantitative variables that are normally distributed), while Mann-Whitney test: for quantitative variables that are not normally distributed. Kruskal-Wallis test: to compare groups for quantitative variables that are not normally distributed followed by post hoc test (Dunn's for multiple comparisons test): for pairwise comparison. Spearman coefficient: to correlate

between quantitative variables. Significance of the results was judged at the level 5%.

Patients were 22 males and one female while control group were 7 males and 3 females with non-significant difference between the patients and control group with ^{FE}p=0.073, ages of the patients ranged between 23 and 55 years old, with mean ± SD of the patients group= 38.6 ± 9.9 and median (IQR)= 37 (30–47), while mean ± SD of the control group ages=35.4 ± 7.7 years and median (IQR)= 34 (30–37) with no significant differences between ages of the patients group and ages of the control group with *p* value= 0.376.

Duration of disease in patients ranged between less than 5 years (10 patients 43.5%), from 5 to 10 years (8 patients 34.8%) and more than 10 years (5 patients 21.7%). Medications that were taken by patients included: steroids (18 patients 69.7% were using steroids with a dosage ranging from 5 to 40 gm per day with mean ± SD=17.2 ± 12.9 gm per day and median (IQR)=15(5–20), 8 patients 34.8% were using cyclosporine with a dose ranging between 100 and 300 mg per day with a mean ± SD= 175 ± 70.7 mg per day and a median (IQR)=200 (100–200), 18 patients 78.3% were using Azathioprine with a dosage ranging between 100 and 150 mg per day with a mean ± SD= 111.1 ±21.4 and a median (IQR)=100 (100–100), 6 patients 18.2% were taking Colchicine 0.5 gm per day twice daily, and only one patient was receiving Hydroxychloroquine 200 mg twice daily and another patient was taking Mesalamine three times a day to relief gastrointestinal symptoms. In 8 patients, 34.8% was having positive HLAB51.

MiRNA548ac expression in the patients group ranged between 0.1 and 12.7 with a mean ± SD = 3.3 ± 4, and a median 1.3 (0.2–5.7) was non-significantly different from control group level ($p = 0.802$), where levels in the control group ranged between 0.2 and 5.2, mean ± SD = 1.6 ± 1.6, median = 1.1 (0.4–1.9). MiRNA548ac expression varied in different clinical groups of the disease but non-significantly as shown in Table 2.

Table 2 Relation between microRNA548ac and different parameters

Sig. bet. grps	*N*	MicroRNA 548ac			*H*	*P*
		Min.–Max.	Mean ± SD.	Median		
		p_1=0.002*, p_2=0.097, p_3=0.202				
Ocular involvement					3.740	0.154
No	**11**	0.07–6.12	1.53 ± 2.16	0.34		
Yes	**12**	0.12–12.68	4.92 ± 4.74	3.50		
Control	**10**	0.16–5.18	1.63 ± 1.62	1.08		
Oral ulcers					2.228	0.328
No	**3**	0.07–4.90	1.70 ± 2.77	0.14		
Yes	**20**	0.12–12.68	3.54 ± 4.21	1.46		
Control	**10**	0.16–5.18	1.63 ± 1.62	1.08		
Genital ulcers					0.200	0.905
No	**4**	0.14–7.64	3.21 ± 3.71	2.54		
Yes	**19**	0.07–12.68	3.32 ± 4.21	1.27		
Control	**10**	0.16–5.18	1.63 ± 1.62	1.08		
Skin lesions						
No	**8**	0.07–12.68	4.42 ± 4.62	3.09	0.860	0.651
Yes	**15**	0.12–12.50	2.70 ± 3.73	0.91		
Control	**10**	0.16–5.18	1.63 ± 1.62	1.08		
Rheumatologic manifestations						
No	**10**	0.12–7.64	2.09 ± 2.93	0.39	3.581	0.167
Yes	**13**	0.07–12.68	4.23 ± 4.63	1.80		
Control	**10**	0.16–5.18	1.63 ± 1.62	1.08		
Vascular events						
No	**14**	0.07–12.68	4.18 ± 4.59	1.72	1.688	0.430
Yes	**9**	0.12–7.64	1.93 ± 2.72	0.34		
Control	**10**	0.16–5.18	1.63 ± 1.62	1.08		

H H for Kruskal Wallis test, Pairwise comparison bet. each 2 groups was done using post hoc test (Dunn's for multiple comparisons test), *p p* value for association between different categories

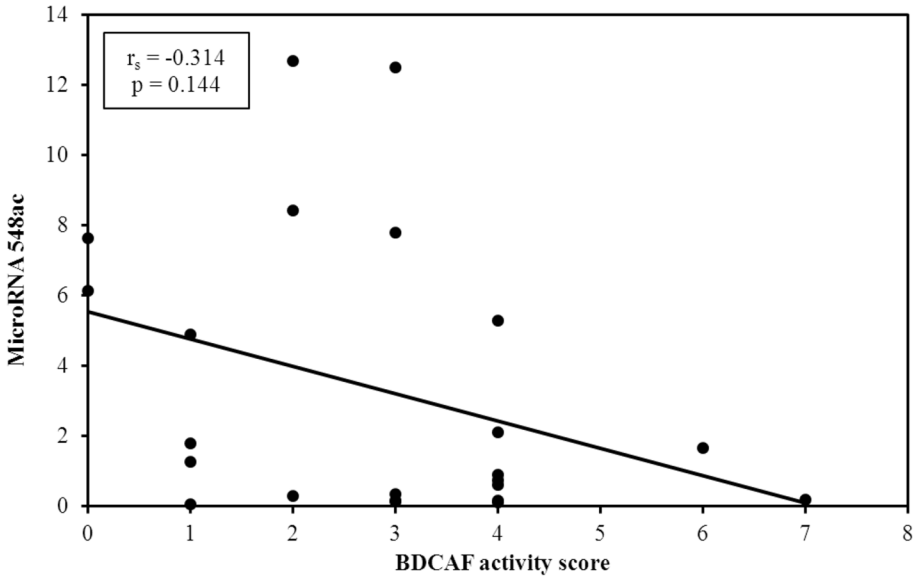

Fig. 1 Correlation between MicroRNA 548ac and BDCAF activity score in total sample

MiRNA548ac was negatively correlated to BDCAF activity score in patients but non-significantly, with rs = −0.314, $p = 0.144$, Fig. 1.

There was significant positive correlation between miRNA548ac expression in the patients and their history of good treatment response, with rs = 0.474, $p = 0.022$, Fig. 2.

Discussion

BD, its consequences, and complications that may lead to disabling or death of some patients and lack of sensitive diagnostic lab tests for the disease make it important to search for a biomarker for disease diagnosis or prognosis.

Previous studies on miRNAs' levels in BD have shown that some miRNAs are increased and some are decreased in the disease, and this may help in follow-up of the disease and knowledge of more about its pathogenesis [15–19].

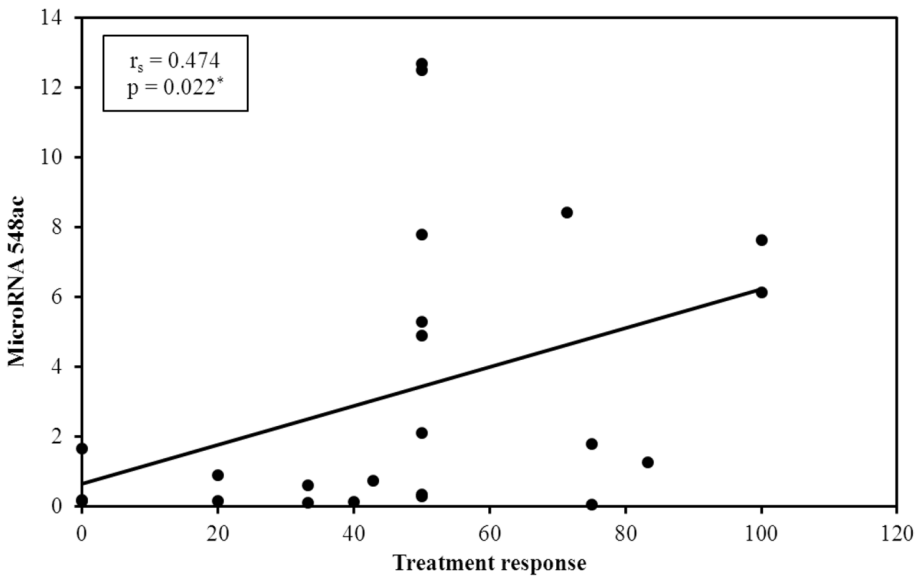

Fig. 2 Correlation between MicroRNA 548ac and treatment response in total sample

Our results showed a decrease in miRNA548ac expression in some but not all BD patients. Our results were not significant in relation to disease activity although evidences that microRNA548ac play a role in autoimmune and auto-inflammatory diseases. As mentioned before, BD is an autoimmune auto-inflammatory disease and this is why this microRNA was investigated in this study.

Th1 was noticed to play a role in BD pathogenesis as mentioned in Tong et al. [20] and Il13 when measured in BD in Aridogan et al. (2003) study, it was increased [21]. IFN-λ1 appeared to have a role in Th1 and Th2 cell development and decreases the secretion of Il13 as what was shown in Jordan et al. (2007) and Darwish et al. (2006) [22, 23], MiRNA548 when tested in Li 2012, it was found that it decreases antiviral response of the host by IFN-λ1 direct targeting [6].

Also expression of miRNA548ac level is decreased in multiple sclerosis (MS) mentioned in Hecker al. 2019 [24], and there is an evidence that MS have an autoimmune element in its pathogenesis as what was mentioned in Wekerle 2005 [5].

In Son et al. (2019), miR-548 was significantly decreased in amnion membranes of patients with chorioamnionitis and this decreased miRNA548 expression upregulated high mobility group box 1 (HMGB1) expression and release from human amniotic epithelial cells (hAECs), and (HMGB1) has a role in the inflammation of spontaneous preterm birth [25]. It is known that BD is considered an auto-inflammatory disease [3], this agrees with the finding in Song et al. (2019) study.

Genc et al. (2018) studied miRNA548b 5p, miRNA548c 5p, and miRNA548i expression levels in subacute sclerosing panencephalitis (SSPE); a neurodegenerative disease caused by persistent defective measles virus infection; and expression levels were found to be significantly higher in SSPE. Increased levels of IL-29 were seen in patients, which indicated that increased miRNA548 expression is a compensatory mechanism for over-activated immune response [26].

In Xing et al. (2014) study [27], it was discovered that one of miRNA548 family (miRNA548ah) and IFN-γR1 mRNA are negatively correlated in levels which is associated with change of immune tolerance to immune activation of chronic hepatitis B (CHB). In some studies, IFN-γ was found to be increased in BD like in (Bacon et, al.1984) study [28].

Another study on miRNA548 in CHB of Yu et al. (2017) [29] found that there is an increase of miNA548j production in CHB, and this leads to decreased IFN-α/β which gives the assumption opposite to our study results that increased miRNA548 production may lead to BD activation and IFN-α was found to treat BD not to activate it as in study of Alpsoy et al. (2002) [30, 31].

MiRNA548 family has shown to be inducing apoptosis when increased in laryngeal tumor mentioned in Song et al. (2020) study [32], while it has shown increase in oesophageal squamous cell carcinoma size and invasion through downregulation of nuclear receptor interacting protein (NRIP1) as what was shown in Ni et al. (2018) study [33]. Regarding BD, resistance to lymphocyte apoptosis has been shown in many studies of BD pathogenesis, e.g., Fujimori et al. (2008), Yang et al. (2002), Hattori et al. (2005), and Torado et al. (2005) [34–36], but there was a study of Giriș et al. (2017) showing that apoptosis of neural cells might be happening in BD [37].

Our study showed did not show the significance of miRNA548ac involvement in disease activity like what was shown in the previous examples of other some inflammatory conditions, but our results showed an increased expression in patients with history of better history of treatment response, so miRNA548ac may predict better treatment response, and some microRNAs were having the same impact as in psoriatic arthritis [8].

Conclusions

MicroRNA548ac did not show a relation to disease activity but this needs further studies; however, it may predict response to treatment; in patients having higher expression of microRNA548ac, a better response to treatment might be predicted. So microRNA548ac could be used for disease prognosis assessment.

This research needs to be done on a larger sample of BD patients and on other autoimmune and auto-inflammatory diseases. It may predict treatment response of the diseased patient.

Abbreviations

BD: Behçet's disease; MicroRNAs (miRNAs): Microribonucleic acid; RNA: Ribonucleic acid; mRNA: Messenger ribonucleic acid; BDCAF: Behçet's Disease Current Activity Form.

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Authors' contributions

SSH chose the miRNA number and the way to measure history of treatment response, collected data and samples from patients, wrote the manuscript and is the corresponding author. MYT who had the idea to measure a miRNA in BD referred some patients to be involved in the study. AIE had the idea of the correlation of the measurement with treatment response. RMA referred patients from the ophthalmology outpatient clinic and has done opthalmological examination of most of the included patients. MT made the laboratory work for measuring the miRNA. The authors have read and approved the manuscript.

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Availability of data and materials

The raw data is available on request.

Declarations

Ethics approval and consent to participate

Ethics committee approval was obtained from the Local Alexandria University Ethics Committee, serial number 0304938, Chairperson: Dr. Maha Ghanem, IRB No. 00012098-FWA, No. 00018699, on December 17, 2020. Verbal informed consent was taken, and the ethics committee approved for that ad provided a permission for the study.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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