Clinical Rheumatology (2019) 38:397–402
https://doi.org/10.1007/s10067-018-4191-2

ORIGINAL ARTICLE

MicroRNA-146a expression and microRNA-146a rs2910164 polymorphism in Behcet's disease patients

W Ibrahim[1] · B. R Sakr[2] · E Obaya[1] · H Ghonem[1]

Received: 13 April 2018 / Revised: 20 June 2018 / Accepted: 24 June 2018 / Published online: 10 July 2018
© International League of Associations for Rheumatology (ILAR) 2018

Abstract

Behcet's disease is a chronic, multisystem, inflammatory disease of unknown etiology. Oral ulcers, genital ulcers, cutaneous lesions, and ocular and articular involvement are the prominent features of the disease. The aim of the study was to assess expression of microRNA-146a and its gene polymorphism in Egyptian Behcet's disease (BD) patients and to evaluate their possible relation with clinical manifestations and activity. This is a case-control study, included 47 BD Egyptian patients, recruited from the Rheumatology outpatient clinic, Kasr Alainy Hospital, Cairo University Hospitals, and 50 healthy controls. BD activity was assessed using the BD Current Activity Score. Quantitative expressions of serum microRNA-146a and microRNA-146a rs2910164 SNP genotyping were performed by real-time polymerase chain reaction (RT-PCR). P values < 0.05 were considered statistically significant. Serum microRNA-146a expression was significantly higher in BD patients than in controls (7. 27 ± 4.11, $1.13 \pm .37$) ($P < 0.001$). There was a significant association between microRNA-146a expression and eye activity ($P = 0.033$) and vascular activity ($P = 0.041$). miRNA-146a rs2910164 genotyping revealed that the frequency of CC genotype was higher in controls (12 vs 8.5%) and the frequency of GG genotype of rs2910164 was higher in the BD patients (59.6 vs 24%) ($P = 0.138$). MicroRNA-146a expression in Egyptian BD patients is significantly higher than that in controls; there is significant association between microRNA-146a expression and eye and vascular activity of BD. The frequency of CC genotype of rs2910164 was decreased; frequency of GG genotype of rs2910164 was increased in BD patients as compared to controls, suggesting that GG genotype of rs2910164 confers susceptibility to BD while CC genotype has a protective role against BD development.

Keywords Behcet's disease · Genotype · MicroRNA · Single-nuclear polymorphism

Introduction

Behcet's disease (BD) is an autoimmune disease characterized by recurrent orogenital ulcers, eye affection, and skin lesions [1]. It is more prevalent in Japan, China, Turkey, and the Mediterranean region [2].

Epidemiological data suggest the involvement of both genetic and environmental factors in the development of BD [3–5]. The disease is assumed to arise from an autoimmune response that may be induced by infectious or other environmental agents in genetically susceptible individuals [3, 6]. Four criteria influence the genetic impact on susceptibility to BD: particular geographical distribution, occasions of familial aggregation, correlation with class I HLA antigens (HLA-B51), and polymorphisms in genes that are involved in immune response control [7–10].

MicroRNAs (miRNAs) are small noncoding regulatory RNAs that have a role in the regulation of gene expression posttranscriptionally [11]. They are involved in many physiological processes and have a regulatory role in many cellular processes such as differentiation, proliferation, and apoptosis [12].

Key points
1. MicroRNA-146a expression in Egyptian BD patients is higher than that in controls.
2. MicroRNA-146a expression is associated with eye and vascular activity.
3. GG genotype of rs2910164 confers susceptibility to BD while CC genotype has a protective role against BD development.

✉ B. R Sakr
Sakrbasma@kasralainy.edu.eg

[1] Department of Medical biochemistry and Molecular Biology, Faculty of Medicine, Cairo University, Cairo, Egypt

[2] Rheumatology and Rehabilitation Department, Kasr Al-Ainy Hospital, Cairo University, Cairo, Egypt

As efficient regulators of numerous genes and pathways in the immune system, miRNAs can be involved in inflammatory- and immune-mediated diseases via affecting regulation of their cellular and molecular targets [13].

MicroRNAs are considered as biomarkers for the diagnosis, prognosis, disease activity, and severity of various diseases. The expression pattern of miRNAs indicates the underlying pathophysiological processes that are specific to various disease states. Moreover, miRNAs can be determined in various sources, including tissue samples, blood components, and body fluids [14].

Several studies have demonstrated that polymorphisms of miRNAs may lead to autoimmune or inflammatory diseases [15, 16]. miR-146a, which was associated with inflammation and apoptosis processes, has been widely confirmed to be related to immune diseases such as rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), and psoriasis [17–19].

Previous reports confirmed that single-nucleotide polymorphisms (SNPs) of miR-146a can alter the expression of mature miRNA-146a [20].

A miR-146a variant, rs2910164, was detected to be strongly associated with BD in a Chinese population. Expression of miR-146a was downregulated, and certain pro-inflammatory cytokines in individuals carry the rs2910164 CC genotype [21].

Patients and methods

Study design: case-control study

Forty-seven BD patients were included in the study and diagnosed according to the International Study Group for BD criteria, 1990. BD patients were recruited from the Rheumatology outpatient clinic, Kasr Alainy Hospital, Cairo University Hospitals, during the period from October 2015 to May 2016. Disease activity was assessed using the BD Current Activity Score (BDCAF) [23]. Fifty age- and sex-matched, healthy subjects were enrolled as controls.

Ethical approval: All procedures performed were in accordance with the ethical standards of Kasr Alainy Cairo University Hospitals research committee and with the 1964 Helsinki declaration ethical standards. Ethics consent and permissions were obtained from participants; consent to publish was obtained from participants.

All patients included in this study were subjected to full history taking, clinical examination, and routine laboratory investigations.

Blood sampling

Five-milliliter peripheral venous blood samples were withdrawn from patients and control subjects and then divided into two parts: 3 ml of blood was allowed to clot and then centrifuged at 8000×*g* for 5 min to separate the serum which was stored at − 80 °C until used for miRNA analysis. A second portion of whole blood (2 ml) was collected in EDTA vacutainer tubes for DNA extraction for genotyping of the studied SNP.

MicroRNA-146a gene expression by real-time polymerase chain reaction

MicroRNA was extracted from serum samples using mirVana kit, USA; then, quantification of miRNA-146a was determined by using the TaqMan® MicroRNA Assays. This is done using two-step RT-PCR: In the reverse transcription (RT) step, cDNA is reversely transcribed from RNA samples using specific micRNA primers from the TaqMan® MicroRNA Assays and reagents from the TaqMan® MicroRNA Reverse Transcription Kit. In the polymerase chain reaction (PCR) step, PCR products are amplified from cDNA samples using the TaqMan® MicroRNA Assay together with the TaqMan® Universal PCR Master Mix. The samples are normalized to miR-16. The assays were performed on step-one real-time PCR system (Applied Biosystems). Relative expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method.

Detection of miRNA-146 gene polymorphism by TaqMan SNP Genotyping Assay

DNA extraction DNA was extracted from the peripheral blood using Qia-amplification DNA extraction kit supplied by Qiagen, USA. The extracted DNA concentration was read at 260 nm on a UV-spectrophotometer supplied by Beckman, USA. The extracted DNA was detected immediately or stored at – 21 °C.

Genotyping miRNA-146a rs2910164 SNP Genotyping was performed using real-time PCR with TaqMan allelic discrimination assay (Applied Biosystems, USA). A predesigned primer/probe sets for the genotypes were used (Applied Biosystems, USA). Real-time PCR was performed with the following conditions: after a denaturation time of 10 min at 95 °C, 45 cycles at 92 °C for 15 s then 60 °C for 90 s for annealing and extension were carried out and fluorescence was measured at the end of every cycle and at the endpoint.

Statistical analysis

Data were coded and entered using the statistical package SPSS (Statistical Package for the Social Sciences) version 24. Data were summarized using mean, standard

Table 1 Description of patients and control data regarding age and sex

		Cases ($n = 47$)	Control ($n = 50$)	P value
Age (years)		34.36 ± 10.95	30.88 ± 6.22	0.198
Sex (%)	Male	85.1% (40)	82% (41)	0.680
	Female	14.9% (7)	18% (9)	

deviation, median, minimum, and maximum in quantitative data and using frequency (count) and relative frequency (percentage) for categorical data. Comparisons between quantitative variables were done using the non-parametric Kruskal-Wallis and Mann-Whitney tests [24]. For comparing categorical data, chi-square (χ^2) test was performed. Exact test was used instead when the expected frequency is less than 5 [24]. Genotype and allele frequencies were compared between the disease and the control groups using chi-square tests. Odds ratio with 95% confidence intervals was calculated. P values < 0.05 were considered to be statistically significant.

Results

General characteristics

The present study included 97 subjects that were divided into two groups: Bechet's disease patients ($n = 47$) and healthy control subjects ($n = 50$). Age of patients ranged from 16 to 74 with a mean of 34.36 ± 10.95 years, while in the control subjects, it ranged from 21 to 45 with a mean of 30.88 ± 6. 22 years. Patients group included 40 (85.1%) males and 7 (14.9%) females and the healthy control group included 41 (82%) males and 9 (18%) females. There were no significant differences between the Bechet's disease patients and control group regarding age and sex ($P = 0.198$, $P = 0.680$) respectively (Table 1). Disease duration ranged from 1 to 34 years with a mean of 8.89 ± 7.56 years. According to BDCAF score, eye activity was detected in 15 (31.9%), central nervous system (CNS) activity in 2 (4.3%), and vascular activity in 4 (8.5%) patients. The clinical manifestations of our patients are shown in Table 2.

Table 2 Clinical characteristics of the studied BD patients

Disease characteristics	Frequency No. (%)
Oral ulcers	45 (95.7)
Genital ulcers	41 (87.2)
Erythema nodosum	12 (25.5)
Pseudo-folliculitis	8 (17)
Arthralgia	18 (38.3)
Arthritis	13 (27.7)
Bleeding/rectum	1 (2.1)
Diarrhea	3 (6.4)
Posterior uveitis	24 (71.8)
CNS	11 (24.4)
Vascular	16 (34)

CNS central nervous system

Table 3 Laboratory findings of the studied BD patients and control subjects

Parameter	Cases	Control	P value
ESR	22.53 ± 18.56	7.40 ± 2.84	< 0.001*
HB	13.07 ± 1.75	13.69 ± 1.36	0.093
TLC	8.64 ± 3.71	7.28 ± 1.98	0.151
PLAT	268.32 ± 83.60	236.51 ± 55.31	0.043*
ALT	35.27 ± 44.93	27.62 ± 12.06	0.588
AST	24.70 ± 12.97	27.70 ± 11.05	0.110
CREAT	0.80 ± 0.16	0.87 ± 0.19	0.128

* = significant p value

As regards the laboratory findings of BD patients and control subjects, Table 3 shows that there was a significant increase in mean levels of ESR and PLA in BD patients compared to the control group ($P < 0.05$).

MicroRNA-146a expression in the studied BD patients and control subjects

Our results showed that microRNA-146a expression was significantly higher in BD patients than in controls (7.27 ± 4.11, 1.13 ± .37 respectively) ($P < 0.001$) (Fig. 1). There was no statistically significant difference between males and females regarding miRNA-146a expression ($P = 0.754$).

There was a significant association between miRNA-146a expression and eye manifestations (posterior uveitis) ($P = 0.009$), CNS manifestations ($P = 0.042$), and both eye activity ($P = 0.033$) and vascular activity ($P = 0.041$) of BD (Table 4.)

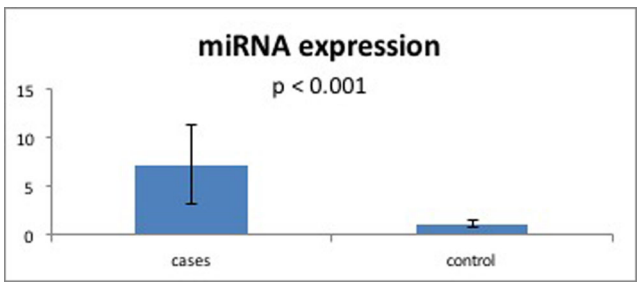

Fig. 1 MicroRNA expression in patients and controls

Table 4 Association of miRNA-146a expression in BD patients with clinical manifestations and disease activity

Clinical manifestations	miRNA-146a expression (mean ± SD)	*P* value
Oral ulcers	7.30 ± 4.11	0.916
Genital ulcers	7.28 ± 4.24	0.962
Skin lesions	7.63 ± 3.68	0.710
Arthralgia	7.99 ± 3.77	0.406
Arthritis	8.35 ± 3.36	0.347
GIT	8.88 ± 2.92	0.505
CNS	9.43 ± 3.57	0.042*
Vascular	6.46 ± 4.07	0.281
Posterior uveitis	8.90 ± 4.00	0.009*
Disease activity		
Eye activity	9.29 ± 3.57	0.033*
CNS activity	11.00 ± 2.27	0.188
Vascular activity	3.41 ± 2.12	0.041*

GIT gastro-intestinal tract, *CNS* central nervous system. *Significant ($P < 0.05$)

Allele and genotype frequencies of SNPs in patients and control

The frequency of the G allele was higher in patients (75.5%) than in controls (56%) ($P = 0.004$) and the frequency of the C allele was higher in controls (44%) than in patients (24.5%). The frequency of CC genotype of rs2910164 was higher in controls than in patients (12 vs 8.5% respectively), while the frequency of GG genotype of rs2910164 was higher in the BD patients than in control subjects (59.6 vs 24% respectively) ($P = 0.138$) (Table 5). There was no association between microRNA (rs2910164) genotypes in BD patients and clinical manifestations of the disease or disease activity (Table 6).

MicroRNA-146a expression and microRNA rs2910164 genotypes

We also investigated the association of this SNP with the microRNA expression and found that the mean was 7.69 ± 4.36 in GG genotype, 7.46 ± 3.71 in GC genotype, and 3.62 ± 2.18 in CC genotype in BD patients ($P = 0.155$), while the mean was 1.41 ± 0.63 in GG genotype, 1.05 ± 0.17 in GC genotype, and 1.02 ± 0.05 in CC genotype ($P = 0.137$) in the control subjects (Figs 2 and 3).

Table 6 Relation of miRNA-146a (rs2910164) genotypes with different parameters

146a genotype		GG (28)		CG (15)		CC (4)		*P* value
		Count	%	Count	%	Count	%	
Oral ulcers		27	92.9	15	100.0	4	100.0	0.611
Genital ulcers		25	89.3	13	86.7	3	75.0	0.524
Skin lesions		12	42.9	5	33.3	1	25.0	0.735
Arthralgia		11	39.3	7	46.7	0	0	0.286
Arthritis		8	28.6	4	27.7	1	25.0	1
GIT		2	7.1	2	13.3	0	0	0.724
Sex	Male	24	82.1	13	86.7	4	100.0	1
	Female	5	17.9	2	13.3	0	0.0	
Eye		21	71.4	9	60.0	3	75.0	0.794
CNS		8	28.6	3	21.0	0	0	0.665
Vascular		12	42.9	4	27.7	0	0	0.233
Eye activity		10	35.7	4	27.7	1	25.0	0.890
CNS activity		1	3.6	1	6.7	0	0	1
Vascular activity		3	10.7	1	6.7	0	0	1

Discussion

To our knowledge, this is the first study investigating the mirocRNA-146a expression and miRNA-146a rs2910164 polymorphism in Egyptian BD patients together with the assessment of their possible association with clinical manifestations and disease activity.

In the present study, the results showed that the overall frequencies of CC, CG, and GG allele combinations were 8.5, 31.9, and 59.6% in the patients vs 12.0, 64.0, and 24.0% in the controls; this is comparable to the results

Table 5 miRNA-146a (rs2910164) genotypes and alleles in the studied groups

		Cases (47)		Control (50)		*P* value	OR (95% CI)
		Count	%	Count	%		
miRNA-146a genotype	GG	28	59.6	12	24.0	0.138	3.5 (0.834–14.69)
	CG	15	31.9	32	64.0	0.717	0.703 (0.172–2.868)
	CC	4	8.5	6	12.0		
	Allele G	71	75.5	56	56	0.004*	2.425 (1.313–4.482)
	Allele C	24	24.5	44	44		

* = significant *p* value

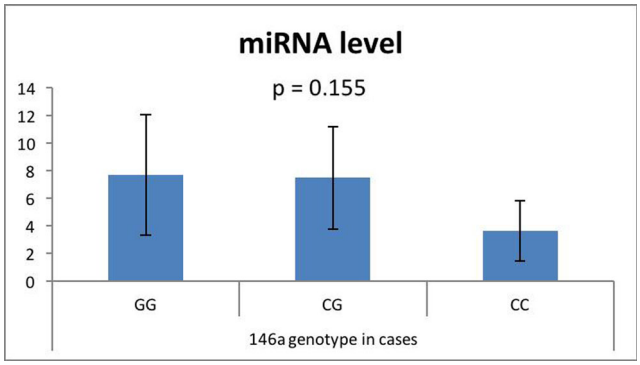

Fig. 2 MicroRNA expression in cases

obtained by Oner and his colleagues [25] who reported that the overall frequencies of CC, CG, and GG allele combinations in BD patients were 1, 33, and 66%, while it was 6, 55, and 39% in the control group.

The frequency of CC genotype of rs2910164 was higher in controls than in patients (12 vs 8.5% respectively), while the frequency of GG genotype of rs2910164 was higher in the BD patients than in control subjects (59.6 vs 24% respectively) ($P = 0.138$). In concordance with our results, Oner et al. [25] stated that the frequency of CC genotype of rs2910164 was higher in the control group than in BD patients, which could suggest a protective role of CC genotype of miR-146a rs2910164 in BD.

Indeed, Zhou et al. [22], who genotyped BD patients for miRNA-146a/rs2910164, rs57095329, and rs6864584, using a PCR assay in Chinese population, reported that miR-146a expression in GG cases was higher in BD patients than that in CC cases, thus concluded that carrying CC genotype of miRNA-146a rs2910164 could attribute to lowering the risk of developing BD.

Our data shows that there was no association between microRNA (rs2910164) genotypes in BD patients and clinical manifestations of the disease or disease activity; these results are matching those obtained by Zhou and his colleagues [22].

The mean expression of microRNA 146 in GG cases was higher than that in CC cases (7.69 ± 4.36, 3.62 ± 2.18 respectively) ($P = 0.155$), which is matching with Jazdzewski et al. [26] and Xu et al. [27].

Our data demonstrates that there was a significant association between miRNA-146a expression and eye manifestations (posterior uveitis) ($P = 0.009$), CNS manifestations ($P = 0.042$), and both eye activity ($P = 0.033$) and vascular activity ($P = 0.041$) of BD, suggesting that BD with higher miRNA-146a expression is more susceptible to eye and vascular activity which should be targeted upon in their regular follow-up.

Our study has several limitations. First, we could not collaborate this study on a larger number of patients due to financial reasons; however, we recommend that in future studies, a big cohort of patients should be conducted to study the effect of miR-146a (rs2910164) polymorphism on the expression of miR146a on the whole population and hence on the prognosis and treatment of BD patients. Second, there were a limited number of previous comparative studies.

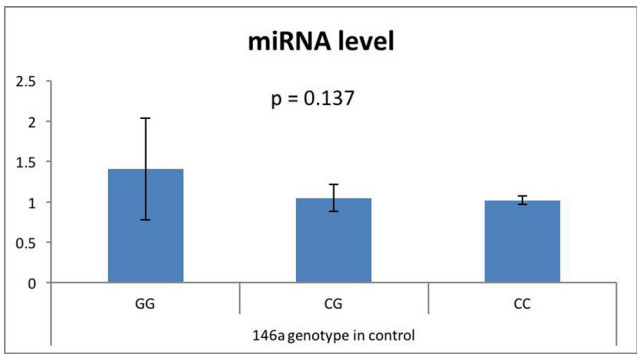

Fig. 3 MicroRNA expression in control subjects

Conclusion

In the present study, miRNA-146a expression in a cohort of Egyptian BD patients is significantly higher than that in control subjects; there is significant relation between miRNA-146a expression, CNS manifestations, and both eye and vascular activity of BD. The frequency of CC genotype of rs2910164 was decreased in BD patients as compared to controls, while the frequency of GG genotype of rs2910164 was higher in the BD patients than in control subjects, suggesting that GG genotype of rs2910164 is associated with the development of BD and the protective role of the CC genotype.

Compliance with ethical standards

Disclosures None.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

Abbreviations BD, Behcet's disease; BDCAF, BD Current Activity Score; CNS, central nervous system; micRNA, micro ribonucleic acids; PCR, polymerase chain reaction

References

1. Ideguchi H, Suda A, Takeno M, Ueda A, Ohno S, Ishigatsubo Y (2011) Behçet disease: evolution of clinical manifestations. Medicine (Baltimore) 90(2):125–132

2. Keino H, Okada AA (2007) Behçet's disease: global epidemiology of an Old Silk Road disease. Br J Ophthalmol 91(12):1573–1574
3. Sakane T, Takeno M, Suzuki N (1999) Behçet's disease. N Engl J Med 341(17):1284–1291
4. Gül A, Inanç M, Ocal L, Aral O, Koniçe M (2000) Familial aggregation of Behçet's disease in Turkey. Ann Rheum Dis 59(8):622–625
5. International Study Group for Behçet's disease (1990) Criteria for diagnosis of Behçet's disease. Lancet 335(8697):1078–1080
6. Mendes D, Correia M, Barbedo M, Vaio T, Mota M, Gonçalves O, Valente J (2009) Behçet's disease—a contemporary review. J Autoimmun 32(3–4):178–188 2
7. Verity DH, Marr JE, Ohno S, Wallace GR, Stanford MR (1999) Behçet's disease, the Silk Road and HLA-B51: historical and geographical perspectives. Tissue Antigens 54(3):213–220
8. Yazici H, Akokan G, Yalçin B, Müftüoğlu A (1977) The high prevalence of HLA-B5 in Behçet's disease. Clin Exp Immunol 30(2):259–261
9. de Menthon M, Lavalley MP, Maldini C, Guillevin L, Mahr A (2009) HLA-B51/B5 and the risk of Behçet's disease: a systematic review and meta-analysis of case-control genetic association studies. Arthritis Rheum 61(10):1287–1296
10. Ohno S, Ohguchi M, Hirose S, Matsuda H, Wakisaka A, Aizawa M (1982) Close association of HLA-Bw51 with Behçet's disease. Arch Ophthalmol 100(9):1455–1458
11. Bartel DP (2004) MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 116(2):281–297
12. Ambros V (2004) The functions of animal microRNAs. Nature 431(7006):350–355
13. Singh RP et al (2013) The role of miRNA in inflammation and autoimmunity. Autoimmunity Rev 12:1160–1165
14. Alevizos I, Illei GG (2010) MicroRNAs as biomarkers in rheumatic diseases. Nat Rev Rheumatol 6:391–398
15. Chatzikyriakidou A, Voulgari PV, Georgiou I, Drosos AA (2012) miRNAs and related polymorphisms in rheumatoid arthritis susceptibility. Autoimmunity Rev 11:636–641
16. Singh S, Rai G, Aggarwal A (2014) Association of microRNA-146a and its target gene IRAK1 polymorphism with enthesitis related arthritis category of juvenile idiopathic arthritis. Rheumatology Int 34:1395–1400
17. Chen HF, Hu TT, Zheng XY, Li MQ, Luo MH, Yao YX, Chen Q, Yu SY (2013) Association between miR-146a rs2910164 polymorphism and autoimmune diseases susceptibility: a meta-analysis. Gene 521:259–264
18. Ji J, Cha E, Lee W (2014) Association of miR-146a polymorphisms with systemic lupus erythematosus: a meta-analysis. Lupus 23: 1023–1030
19. Zhang W et al (2014) A single-nucleotide polymorphism of miR-146a and psoriasis: an associationand functional study. J Cellular Molecular Med 18:2225–2234
20. Luo X, Yang W, Ye DQ, Cui H, Zhang Y, Hirankarn N et al (2011) A functional variant in microRNA-146a promoter modulates its expression and confers disease risk for systemic lupus erythematosus. PLoS Genet 7(6):e1002128
21. Zhou Q, Hou S, Liang L, Li X, Tan X, Wei L et al (2014) MicroRNA-146a and Ets-1 gene polymorphisms in ocular Behcet's disease and Vogt-Koyanagi-Harada syndrome. Ann Rheum Dis 73(1):170–176
22. Bhakta BB, Brennan P, James TE, Chamberlain MA, Noble BA, Silman AJ (1999) Behçet's disease: evaluation of a new instrument to measure clinical activity. Rheumatology (Oxford) 38(8):728–733
23. Chan YH (2003) Biostatistics 102: quantitative data—parametric & non-parametric tests. Singap Med J 44(8):391–396
24. Chan YH (2003) Biostatistics 103: qualitative data—tests of independence. Singapore Med. J 44(10):498–503
25. Oner T, Yenmis G, Tombulturk K, Cam C, Kucuk OS, Yakicier MC, Dizman D, Sultuybek GK (2015) Association of pre-miRNA-499 rs3746444 and pre-miRNA-146a rs2910164 polymorphisms and susceptibility to Behcet's disease. Genet Test Mol Biomarkers 19(8):424–430
26. Jazdzewski K, Murray EL, Franssila K, Jarzab B, Schoenberg DR, de la Chapelle A (2008) Common SNP in pre-miR-146a decreases mature miR expression and predisposes to papillary thyroid carcinoma. Proc Natl Acad Sci U S A 105(20):7269–7274
27. Xu T, Zhu Y, Wei QK, Yuan Y, Zhou F, Ge YY et al (2008) A functional polymorphism in the miR-146a gene is associated with the risk for hepatocellular carcinoma. Carcinogenesis 29(11):2126–2131