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Expression Levels of lncRNA NEAT1, miRNA-21, and IL-17 in a Group of Egyptian Patients with Behçet's Disease: Relation to Disease Manifestations and Activity

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Background: Long noncoding ribonucleic acids (lncRNAs), small noncoding RNAs known as microRNAs (miRNAs) as well as some cytokines are recently thought to have a role in many inflammatory and autoimmune disorders including Behçet's disease (BD). This chronic multisystem disease lacks the particular histological or laboratory findings that might aid in its diagnosis. Therefore, any association with such molecules may have an impact on understanding the disease pathogenesis and/or management. The current study compared the levels of NEAT1, miR-21 and IL17 levels in sera of Egyptian BD patients and healthy individuals. The expression levels of these molecules were further investigated for their association with BD manifestations and activity aiming to explore their potential application in disease management.

Results: NEAT1 & miR-21 showed down-regulation while IL-17 showed up-regulation among BD patients as compared to controls. IL-17 had significant correlation with major vessels involvement and cyclophosphamide intake. NEAT1 showed a significant negative correlation with colchicine intake. Disease activity did not correlate significantly with any of NEAT1, miR-21 or IL-17.

Conclusion: NEAT1, miR-21 and IL17 might have a role in Behçet's disease pathogenesis, so more research is needed to unveil that role and their potential usage as biomarkers for the diagnosis or therapeutic targets.

Keywords: long noncoding ribonucleic acids, microRNAs, NEAT1, miR-21, IL-17, Behçet's disease

Introduction

Behçet's disease (BD) is an uncommon chronic multisystem disease for which there are no particular histological or laboratory findings that have been found to aid in the diagnosis; rather, Behçet's syndrome is defined and diagnosed based on clinical criteria which have been changed lately by an international study group.[1,2]

The pathogenetic mechanisms underlying BD are not fully elucidated. Growing evidence suggests an important role for some of the long noncoding ribonucleic acids (lncRNAs) in many inflammatory and autoimmune disorders including BD.[3] These genes, in which 200 nucleotides accumulate, were historically considered the dark matter of the genome due to lacking biological functions such as encoding for proteins. However, they are being recently widely studied to explore their potential roles in many inflammatory disorders. For instance, nuclear paraspeckle assembly transcript 1 (NEAT1) has been linked to different inflammatory and immune responses such as cancer, viral infection and neurodegeneration.[4]

Received: 30 August 2024
Accepted: 6 November 2024
Published: 12 November 2024

Some investigators suggested that NEAT1 may play a role in BD pathogenesis through controlling the secretion of several chemokines and cytokines such as IL-6.[5]

MicroRNAs (miRNA), on contrast, are small, single-stranded, non-coding RNA molecules that are 19–20 nucleotides in length.[6] MiRNAs interact with cellular messenger RNAs to regulate a number of biological processes.[7] MiRNA synthesis and expression is impacted by inflammatory responses, through modifying precursor transcript transcription and processing or stabilizing mature miRNAs.[8,9] The number of miRNAs linked to immune system development and function has increased significantly in recent years. This led to extensive research regarding their potential application in diagnosis and treatment of immunological diseases.[7] MiR-21 was one of the first recognized and transcribed miRNAs. It was found to be involved in several conditions including cancers, cardiovascular, inflammatory and immunological processes.[10] The expression pattern of miR-21 was noted to influence the differentiation of CD4+ T cells into Th1, Th2, or Th17 responses. In BD, it has been observed that miR-21 has a lower level than in normal population; however, it was positively associated with severe eye involvement.[11]

T helper 17 lymphocytes (Th17) secrete interleukin 17 (IL-17), a proinflammatory cytokine that is believed to be involved in many chronic inflammatory and autoimmune processes.[12] Th17 might be crucial to the etiology of BD.[13,14] The discovery of elevated IL-21 and IL-17 levels in the sera of BD patients with neurologic dysfunction lends credence to this theory.[15,16] Individuals with BD had greater Th17/Th1 ratios in their peripheral blood when compared to healthy controls. Individuals who also had uveitis or folliculitis had higher Th17/Th1 ratios than those who did not have these symptoms.[17,18]

We conducted the current study to compare the serum levels of NEAT1, miR-21 and IL17 in a cohort of Egyptian BD patients with those in normal individuals, and to investigate their correlation to various BD manifestations and disease activity as measured by The Behçet's Disease Current Activity Form (BDCAF) and the Behçet's Syndrome Activity Score (BSAS) to see if they could have a potential application as biomarkers or therapeutic targets for the BD.

Materials and Methods

Type and Setting of the Study

This is an analytical, observational case- control study, conducted on two groups of individuals. A group of adult BD patients (age >18 years, No. =51) recruited from outpatient clinics, Kasr Al-Ainy Faculty of Medicine, Cairo University hospital, during the period from 1-6-2023 to 31–12-2023. They were fulfilling The International Criteria for Behçet's Disease (ICBD).[1] The following cases were excluded: 1-Juvenile cases 2- patients with associated other autoimmune diseases 3- pregnant females 4- Patients with other forms of vasculitis or hyper-coagulable states. The second group included age –and sex- matching healthy controls (No. =40) recruited from healthy blood donors visiting the blood bank of the hospital. Full medical history, thorough clinical examination and routine laboratory tests were performed to all included patients. For assessment of BD disease activity, we used both The Behçet's Disease Current Activity Form (BDCAF)[19] and the Behçet's Syndrome Activity Score (BSAS).[20] Sera of patients and controls were collected for testing for IL-17, NEAT1, and miR-21. Biochemical analysis was carried out in the biochemistry and molecular biology unit. All the included subjects signed an informed consent. The study was conducted in adherence to the Helsinki declaration and was approved by the Scientific Research Ethics Committee of the Faculty of Medicine, Cairo University, with number (N-213-2023).

Fold Changes of NEAT1 and miR-21 in Serum

Total RNA extraction from serum (200 µL) was conducted using Qiagen miRNeasy Serum/Plasma kit (Cat. no. 217184), as instructed by the vendor. Extracted RNA yield and purity were measured by a nanodrop (Bioanalyzer Agilent RNA 6000 bioassay). Samples with A260/280 ratio between 1.8 and 2.2 were further used for reverse transcription (RT).

RT was attempted on 100 ng of total RNA in a 20 µL RT reaction mixtures using miScript II RT kit (Cat.no. 218160) (Qiagen) following the manufacturer's protocol. Using GAPDH as an internal control gene, the expression profiles of NEAT1 was determined by qPCR. The GAPDH gene has been validated previously for use as an excellent internal control for normalizing lncRNAs. The qPCR assay was conducted using the Maxima SYBR Green PCR kit

(ThermoFischer, USA) (Catalog numberK0221) following the manufacturer's recommendations. Melting curve analysis was also performed to ensure the absence of primer dimers.

Using the Rotorgene Q system (Qiagen), real-time PCR was implemented in 20 µL reaction mixtures miR-21 expression was done using RT-qPCR. The miScript II RT kit (Qiagen) was used for reverse-transcribing 0.1 µg of total RNA in a 20 µL final volume following the instructions of the manufacturer. The RT was executed using thermal parameters: 60 min at 37 ◦C and 5 min at 95 ◦C. For qPCR, the miScript SYBR Green PCR kit (Qiagen) was employed to prepare a total of 20 µL reaction mixtures of the cDNA with the ready-made miScript reverse (Universal) primer and specific forward primers for hsa-miR-21 and SNORD68 (internal control).

SNORD68 was used as an internal control for miRNA normalization. As SNORD68 expression is stable and consistent across both disease cells and normal cells, it can be used as a reliable reference for estimating relative miRNA levels especially since there is not a known control miRNA in serum. We employed the Rotorgene Q real-time system (Qiagen) with the following PCR thermal conditions: 15 min at 95 ◦C, 40 cycles of 15s at 94 ◦C followed by 30s at 55 ◦C and 30s at 70 ◦C.

The fold change (=relative quantitation to control ie, the amount of gene expression in patients in relation to controls) was calculated for the expression of studied genes using the formula $2^{-\Delta\Delta Ct}$, where ΔΔCt = ΔCt patient – ΔCt control group.

Quantitation of IL-17 in Serum

An enzyme-linked immunosorbent assay (ELISA) kit (by Bioassay Technology Laboratory (Zhejiang, China) was employed to assess IL-17 in serum. Human antibody against IL-17 was previously coated on the plate. After being presented, the sample's IL-17 attaches to the antibodies coated on the wells. Next, biotinylated Human IL-17 Antibody is added, to attach to the sample's IL-17. Then, the biotinylated IL-17 antibody binds to streptavidin-HRP. After incubation, a washing stage eliminates unbound streptavidin-HRP. Then by adding a substrate solution, the color changes in direct proportion to the concentration of human IL-17. The procedure ended by an acidic stop solution, and the absorbance was assessed at 450 nm.

Statistical Analysis

The statistical package for social sciences (SPSS) (IBM SPSS, IBM Corp., Armonk, N.Y., USA) version 22 was employed to analyze the data. Categorized data were expressed as frequency (%), while parametric data were represented by the mean (± standard deviation), or median (minimum – maximum/25-75 percentile) as appropriate. Data distribution normality was tested for using the Shapiro–Wilk Test. Pearson Chi-Square test was used in assessment of categorized data, Mann–Whitney test for abnormally distributed parametric data and unpaired student "t" test for normally distributed parametric data. P-value below 0.05 indicated statistical significance.

Results

The study included 2 groups, patients group which included 51 BD patients and control group which included 40 age- and sex- matched healthy persons. The mean age was 33.27(±7.38) and 35.33(±6.03) years for patients and controls respectively. Males comprised 84.3%of patients and 82.5% of controls, while females were 8% in patients group versus 7% in control group (Table 1).

In the patients' group, individual clinical presentations, comorbidities, medications, and routine labs are mentioned in Table 2. BDCAF patient´s index Score ranged from 2 to 4 (with mean =3), and Behcet Syndrome Activity Score (BSAS) ranged from 16 to 40 (with mean =25) (Table 2).

IL-17 level was significantly higher in patients' group than in controls, while NEAT1 & miR-21 were significantly lower in patients than in controls (Table 3). However, their levels did not correlate significantly with disease duration, or disease activity measured by BDCAF or BSAS scores as mentioned in Table 4.

On further analysis to detect the correlation of NEAT1, miR-21 and IL-17 levels with individual items of the demographic and clinical characteristics of the BD patients, it was noted that only major vessels involvement has correlated significantly with IL-17 level (*p* value 0.034) (Table 5). As regards medications, IL-17 showed a significant positive correlation with cyclophosphamide intake (*p* value 0.048), and NEAT1 showed a significant negative correlation with colchicine intake (*p* value 0.007) (Table 6).

Table 1 Demographic Characteristics of Patients and Control

Characteristics	Behçet's Disease Group (No.=51)	Healthy Control Group (No.=40)	P- value
Age (years)	33.27±7.38 (20–46)	35.33±6.03 (21–43)	0.158
Gender			
Male No. (%)	43 (84.3%)	33 (82.5%)	0.518
Female No. (%)	8 (15.7%)	7 (17.5%)	

Notes: Data expressed as mean ± standard deviation (minimum – maximum) for parametric data, and frequency (%) for categorized data.

Table 2 Comorbidities, Clinical Presentations, Medications and the Routine Laboratory Findings of the Patients

Disease Duration (years)	**3.31±1.50 (1–9)**
Comorbidities	
Hypertension No. (%)	6 (11.8%)
Diabetes mellitus No. (%)	5 (9.8%)
Clinical presentation	
Eye involvement No. (%)	33 (64.7%)
Arthralgia No. (%)	31 (60.8%)
Mouth ulcerations No. (%)	23 (45.1%)
Headache No. (%)	16 (31.4%)
Erythema nodosum No. (%)	14 (27.5%)
Nausea/ vomiting No. (%)	11 (21.6%)
Genital ulceration No. (%)	7 (13.7%)
Skin pastules No. (%)	7 (13.7%)
Major vessels involvement No. (%)	6 (11.8%)
Diarrhea No. (%)	4 (7.8%)
Arthritis No. (%)	3 (5.9%)
New active nervous system involvement No. (%)	2 (3.9%)
Medications	
Steroids No. (%)	37 (72.5%)
Steroid dose mg/day (mean±SD)	32.30±15.97 (5–60)
Colchicine No. (%)	40 (78.4%)
Azathioprin No. (%)	31 (60.8%)
Cyclosporine No. (%)	14 (27.5%)
Cyclophosphamide No. (%)	12 (23.5%)

(*Continued*)

Table 2 (Continued).

Disease Duration (years)	**3.31±1.50 (1–9)**
Warfarin No. (%)	3 (5.9%)
Biologic s No. (%)	2 (3.9%)
Activity assessment	
BDCAF Patient's Index Score (0:12)	3 (2–4)
BSAS score/100	25 (16–40)
Routine laboratory parameters	
Hemoglobin mg/dl	12.04±1.41 (10–16)
WBC No./cc	7.35±2.28 (4–11)
PLT No./cc	265.00 (221.00–321.00)
ESR mm/h	19.00 (10.00–25.00)
AST U/L	23.00 (20.00–31.00)
ALT U/L	24.00 (20.00–30.00)
Albumin g/dl	4.02±0.14 (4–5)
Creatinine mg/dl	1.00±0.00 (1–1)

Notes: Data expressed as mean ± standard deviation (SD) (minimum - maximum) for parametric data, and frequency(%) for categorized data.
Abbreviations: BDCAF, Behcet's Disease Current Activity Form; BSAS, Behcet Syndrome Activity Score; WBC, White blood cells count; PLT, platelets; ESR, erythrocyte sedimentation rate; AST, Aspartate aminotransferase; ALT, Alanine aminotransferase; cc, cubic centimeter; mm/h, millimeter/hour; U/L, unit/liter; g/dl, gram/deciliter; mg/dl, milligram/deciliter.

Table 3 Comparison of NEAT1, miR-21 and IL-17 in Patients and Control

Variable	**Patients (No.=51)**	**Control (No.=40)**	**Significance (*p* value)**
NEAT1	0.08 (0.04–0.15)	1.00 (1–1)	**0.0001***
miR-21	0.10 (0.01–0.047)	1.00 (1–1)	**0.0001***
IL-17 (pg/mL)	106.00 (101.00–124.00)	38.50 (35.00–45.00)	**0.0001***

Notes: Data expressed as median (25–75 percentile). Comparison between groups was made using Mann Whitney test. Bold number means significant. Asterisks (*) means p value <0.05.
Abbreviation: pg/mL, picogram/milliliter.

Table 4 Correlations of NEAT1, miR-21 and IL-17 with BDCAF & BSAS Scores of Behcet's Patients

Variable	**BDCAF Patient's Index Score**		**BSAS Score**		**Disease Duration**	
	R	**P**	**R**	**P**	**R**	**P**
NEAT1	−0.028	0.845	−0.040	0.779	−0.174	0.222
miR-21	0.135	0.346	0.240	0.089	−0.020	0.892
IL-17	0.072	0.617	0.042	0.768	0.049	0.731

Notes: Correlation was made by Spearman test.
Abbreviations: BDCAF, Behcet's Disease Current Activity form; BSAS, Behcet Syndrome Activity Score.

Table 5 Demographic and Clinical Characteristics of BD Patients in Comparison to Levels of NEAT1, miR-21 and IL-17

Characteristics		NEAT1	*P*	miR-21	*p*	IL-17 (pg/mL)	*p*
Gender	Male	0.28 (0.07–1.00)	0.468	1.00 (0.07–1.00)	0.437	69 (39–112.75)	0.087
	Female	0.26 (0.08–1.00)		1 (0.10–1.00)		83 (39–103)	
Headache	No	0.08 (0.04–0.18)	0.984	0.10 (0.04–0.42)	0.976	106 (99–125)	0.879
	Yes	0.11±0.09		5.93±19.91		124.25±67.86	
Mouth ulcerations	No	0.80 (0.3–0.17)	0.532	0.08 (0.02–0.35)	0.268	111.5 (101–124)	0.733
	Yes	0.08 (0.04–0.15)		0.27 (0.01–3.03)		106 (100–124)	
Genital ulceration	No	0.08 (0.04–0.13)	0.419	0.08 (0.01–0.42)	0.053	106 (100.25–123.25)	0.359
	Yes	0.15 (0.5–0.25)		0.42 (0.10–4.32)		107 (103–202)	
Erythema nodosum	No	0.09 (0.05–0.16)	0.635	0.27 (0.03–0.47)	0.410	106 (100.5–121)	0.321
	Yes	0.08 (0.04–0.15)		0.06 (0.01–0.62)		119 (103.75–124.75)	
Skin pustules	No	0.09 (0.05–0.18)	0.352	0.19 (0.01–0.47)	0.435	106.5 (101–124)	0.547
	Yes	0.08 (0.04–0.10)		0.07 (0.01 (0.24)		105 (93–127)	
Arthralgia	No	0.09 (0.05–0.15)	0.817	0.08 (0.01–0.40)	0.311	111.5 (97.25–124)	0.946
	Yes	0.08 (0.04–0.18)		0.24 (0.04–0.48)		106 (101–124)	
Arthritis	No	0.08 (0.04–0.15)	0.719	0.10 (0.01–0.47)	0.810	106 (101–123.25)	0.367
	Yes	0.10 (0.04–0.10)		0.24 (0.13–0.24)		288 (79–288)	
Nausea/ vomiting	No	0.09 (0.05–0.17)	0.325	0.18 (0.02–0.46)	0.422	106 (100–124)	0.722
	Yes	0.07 (0.03–0.14)		0.07 (0.01–0.48)		107 (101–125)	
Diarrhea/ BPR	No	0.08 (0.04–0.15)	0.661	0.10 (0.01–0.47)	0.861	106 (100–124)	0.318
	Yes	0.07 (0.03–0.21)		0.17 (0.03–59.99)		116 (102.5–313.25)	
Eye involvement	No	0.08 (0.03–0.13)	0.391	0.10 (0.12–0.30)	0.265	109 (96–124.25)	0.745
	Yes	0.09 (0.05–0.18)		0.30 (0.01–0.77)		106 (101–124.5)	
New active nervous system involvement	No	0.08 (0.04–0.14)	0.627	0.10 (0.01–0.47)	0.319	106 (100–124)	0.159
	Yes	0.15 (0.06–0.15)		0.14 (0.002–0.14)		244 (112–244)	
Major vessels involvement	No	0.08 (0.05–0.17)	0.327	0.10 (0.01–0.47)	0.918	106 (100–122)	**0.034***
	Yes	0.08±0.08		1.71±3.79		187.00±115.92	

Notes: Data expressed as median (25–75 percentile). Comparison between groups was made using Mann Whitney test. Bold number means significant. Asterisks (*) means p value <0.05.

Abbreviations: BPR, Blood Per Rectum; pg/mL, pictogram/milliliter.

Discussion

The current study was conducted to investigate the expression levels of lncRNA NEAT1, miR-21 and IL-17 in Egyptian BD patients aiming to find a potential implication of these molecules as diagnostic biomarkers or therapeutic targets for the disease. The study revealed that NEAT 1 and miR-21 were significantly lower in BD patients than in controls, meanwhile IL-17 showed a significantly higher expression level among patients. A significant association was found between IL-17 level and major vessels involvement in BD patients. Moreover, IL-17 showed a low positive yet significant association with cyclophosphamide intake in patients with BD, whereas a negative association was observed

Table 6 Correlations of NEAT1, miR-21 and IL17 Levels with Medications Used for BD

Variable	NEAT1		miR-21		IL-17	
	R	p	R	P	r	p
Steroids	0.076	0.595	0.052	0.716	0.167	0.241
Colchicine	**−0.372**	**0.007***	−0.026	0.857	−0.094	0.512
Azathioprine	−0.012	0.932	0.038	0.790	0.093	0.517
Cyclosporine	−0.018	0.901	−0.063	0.662	−0.043	0.763
Cyclophosphamide	0.259	0.066	−0.027	0.852	0.278	**0.048***
Warfarin	−0.110	0.441	0.003	0.984	0.133	0.351
Biologics	−0.144	0.313	0.237	0.094	0.076	0.598

Notes: Correlation was made by Spearman test. Bold number means significant. Asterisks (*) means p value <0.05.

between NEAT1 and colchicine intake. None of the three molecules (NEAT1, miR-21 and IL-17) showed association with BD duration or activity.

Studies on NEAT1 revealed controversial results regarding immunity and inflammation, supposing a dual action of NEAT1. While some studies suggest a pro-inflammatory effect of NEAT 1 in some cancers and autoimmune diseases like Rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE),[21–27] many other researches support an alternative perspective in which NEAT 1 has a protective role against inflammation. For instance, Blockage of NEAT1 enhances inflammation induced by lipopolysaccharides (LPS).[28] NEAT1 can also inhibit the tumor-like features, and release of cytokines in fibroblast like synoviocytes in RA patients.[29] The role of NEAT1 in BD is not clear and only few studies have been conducted to elucidate this role. NEAT1 was found to improve Dendritic cells (DCs) related immune tolerance.[30] DCs are known to have a great role in BD pathogenesis.[31]

Interestingly, NEAT1 was found to be significantly down regulated in our cohort. Moreover, NEAT1 exhibited a negative correlation with colchicine intake. Colchicine was taken by most of our patients, and this might be related to the decreased levels of NEAT1 among patients in comparison to controls. Future studies with larger cohorts from different ethnicities are needed to confirm this hypothesis. The low level of NEAT1 in BD goes hand to hand with the results of a similar study conducted on Egyptian patients with BD.[4] Han et al found a similar negative correlation with colchicine where NEAT1 plasma levels decreased markedly in patients with recurrent active aphthous stomatitis after they received colchicine.[32] We failed to find an association between NEAT1 and BD activity or manifestations, in the opposite to Mohammed et al who found a negative relation between NEAT1 and disease activity.[4]

The factors that influence NEAT 1 to take either pro or anti-inflammatory paths have not yet been identified. Studies had revealed that NEAT1 can achieve its function through targeting miR-21.[33,34] Hence, this study aimed to reveal a relation between NEAT1 and miR-21 in BD.

In the present study, miR-21 showed significant downregulation in patients compared to controls. The same result was reached by Jadideslam et al who found decreased levels of miR-21 in BD patients.[11] MiR-21 levels were also found to be significantly decreased in patients with rheumatoid arthritis.[35] On the other hand miR-21 was reported to be associated with other autoimmune diseases like SLE[36] and psoriasis.[37]

In the current study, miR21 was not correlated with disease activity and manifestations in contrast to another study that found a direct relation with uveitis.[11]

MiR-21 is supposed to affect BD pathogenesis in several ways. It can regulate many signaling pathways, and can contribute to angiogenesis, endothelial cell migration, and differentiation.[38,39] MiR-21 has an antiangiogenic action through affecting the expression of RhoB in the endothelial cells.[40] Angiogenesis and endothelial cell dysfunction play major roles in BD pathogenesis.[41] MiR21 can also regulate the immune response especially T cell response.[42,43] MiR-21

regulates T cell stimulation and apoptosis, Th17, Treg cells function and differentiation, it can also keep Th1/Th2 cell balance.[44] Th1 and Th17 cell amplification together with regulatory T cells (Tregs) damage has been found to be one of the main pathogenetic mechanisms of BD.[45,46] In addition, miR21 can preserve the low expression levels of inactive T cells together with antigen presenting cells (APC). From the mentioned studies, we could predict an inhibitory role of miR-21 on the immune cells including Th17, the producing cells of IL-17. Therefore, we included IL-17 in our work, and indeed observed an inverse relationship between miR-21 and IL-17.

In the current study, measuring IL-17 serum levels in patients and control groups revealed significant up regulation of IL-17 levels in the patients group.

Many studies had investigated the role of IL-17 in BD; one of these studies was conducted on Egyptian BD patients that showed similar results to the current study[47] in addition to other studies that had revealed similar outcomes.[48–50]

Notwithstanding the studies performed on IL-17 in BD, its definite role in BD remains incompletely understood.[51] Hamzaoui et al had investigated the role of IL-17 in BD and found enhancement of Th17 cells regarding their number and ability of IL-17 production.[52] The increased Th17/ Treg cells ratio is involved in BD pathogenesis. These increased levels of Th17 cells, with Th1 cells, could be involved in BD pathogenesis via the IL-23–IL-17 pathway through a direct increase relation between both cytokines especially in disease activity.[48,53] This axis was found to play a role in experimental uveitis in rats where treatment of uveitis could be obtained through blocking the IL23-IL17 pathway.[54] IL-17 was also found to attract neutrophil cells to inflammation sites; enhancing inflammation in BD.[55] Elevated IL-17+ cells was reported in the erythema nodosum and other skin lesions in BD skin.[50,52,56]

In this study, IL-17 showed no correlation with disease activity. Nevertheless, it was significantly elevated in patients with major vessels involvement. Additionally, IL-17 showed a positive correlation with cyclophosphamide intake by BD patients. This finding was supported by other studies that revealed association of IL-17 with major vessel vasculitis[57–59] It remains to be elucidated whether the high expression of IL-17 in serum of BD patients and its correlation with cyclophosphamide intake could be a reflection of the frequent administration of this drug in BD patients with vasculitic element which might indicate a more severe form of the disease or could be a direct effect of cyclophosphamide intake. In a study conducted by Viaud et al, they demonstrated that cyclophosphamide enhanced the differentiation of CD4+ T cells into Th17 resulting in an increased production of IL-17 in rodent models and cancer patients.[60] From the preceding argument, it can be inferred that IL-17 may play a significant role in the pathogenesis of BD, suggesting it could serve as a potential therapeutic target for the disease.

Our study has some limitations to be acknowledged: first, there is scarcity of previous research studies investigating the same molecules in BD. Second: We encountered some financial challenges while conducting the study, which hindered our ability to perform further analysis. Third: the small sample size and the number of controls which may affect the results or exaggerate the findings. Finally, we faced some time limitations. Hence, we recommend future research on a wider scale with large multicenter study populations to validate our findings and reach a strategy for potential applications of these molecules in BD.

Conclusion

In summary, our research showed that NEAT1, miR-21, and IL-17 were differentially expressed in patients with Behçet's disease. NEAT1 was negatively related to colchicine intake. IL-17 was positively related to major vessel affection and cyclophosphamide administration. None of IL-17, NEAT1 or miR-21 correlated significantly with BD activity as measured by BDCAF or BSAS. NEAT1, miR-21 and IL17 might affect BD pathogenesis and whole through course but their definite roles remain to be clarified and further studies with increased number of patients can allow clarification of these roles.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Funding

No funding was received to conduct this study.

Disclosure

The authors report no conflicts of interest in this work.

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