Egypt J Pediatr Allergy Immunol 2022; 20 (2):79-85.

Original article

KAT2B expression on CD4+T lymphocytes in pediatric systemic lupus erythematosus

Background: *The pathogenesis of pediatric systemic lupus erythematosus (pSLE) is multifactorial and includes genetic predisposition and modifiable environmental factors. Lysine Acetyl transferase 2B (KAT2B), is one of the histone acetylases that regulate the gene transcription. It was linked to autoimmune diseases with variable expression in relation to various disease parameters. We sought to investigate the KAT2B expression on peripheral blood mononuclear cells (MNCs) in patients with pSLE and its relation to biomarkers of lupus flare, major organ involvement, SLE disease activity index (SLEDAI), and therapeutic modalities used.* ***Methods:*** *This cross-sectional comparative study comprised 30 patients with SLE who fulfilled at least four of the System Lupus International Collaborating Clinics (SLICC) classification criteria. Thirty age- and sex-matched healthy children were included as a control group. The patients were subjected to clinical evaluation including the SLEDAI, and lupus flare laboratory markers. KAT2B expression on MNCs was measured by ELISA in the pSLE patients as well as the control group.* ***Results:*** *KAT2B expression on the MNCs was significantly lower among the pSLE patients than the healthy controls (p <0.001). Patients with moderate and severe lupus activity had significantly lower KAT2B expression on MNCs than those with mild activity as judged by the SLEDAI (p=0.03). The KAT2B expression was not significantly correlated to the studied biomarkers of lupus activity (ESR, anti-DNA or C3) but was negatively correlated to the extent of renal affection in terms of the 24 hours urinary protein level (p=0.024). The findings are limited by the sample size. Conclusion: From this pilot study, the low expression of KAT2B on MNCs seems to be linked to pediatric SLE disease activity. Wider scale and prospectively designed studies are needed to validate this observation and to explore the effect of disease remission on KAT2B expression in pSLE activity.*

Key words: Lysine Acetyl transferase 2B; KAT2B; pediatrics; SLE; SLEDAI.

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INTRODUCTION

The main pathogenetic key of systemic lupus erythematosus (SLE) is break down of self-tolerance involving both the adaptive and innate immune responses. This results in the production of excessive autoantibodies and complement activation with immune complex deposition in different tissues.[1]

Various studies have shown that epistasis, or gene–gene interaction, has an important role in SLE susceptibility. Also, epigenetic factors such as cytosine methylation, histone modifications, and microRNA profile may play a role in the development and specific phenotype of the disease.[2] It was suggested that epigenetic mechanisms modulate both the innate and adaptive immune responses via differentiation of monocytes/macrophages, dendritic cells, neutrophils, and T-helper cells.[3] Histone acetyltransferases (HATs), including Lysine Acetyltransferase 2B (KAT2B), control gene expression by adding acetyl groups on histone lysine residues. KAT2B, formerly known as PCAF, is a protein coding gene located on chromosome location 3p24.3 in the human genome.[4] It plays a direct role in transcriptional regulation of many histone proteins and non-histone proteins as well.[5] CD8+ T cells become exhausted due to persistent antigen exposure and the absence of CD4+T- cells co-stimulation.[6] Exhausted T cells progressively loose its effector functions (cytokine production and killing function) and express multiple inhibitory receptors such as programmed death (PD-1) and lymphocyte activation gene 3 (LAG3). In addition, it is characterized by abnormal metabolism, poor memory recall, and homeostatic proliferation.[7] In patients with SLE, some co-

inhibitory receptors characterize exhausted T cells, which is translated clinically into a non-relapsing course. The exhausted phenotype can be rescued by co-stimulation, and certain genes, like KAT2B, can facilitate this. Moreover, KAT2B is anti-apoptotic and mediates protection against metabolic stress. In order to decrease disease relapses, therapeutic interventions that increase T cell exhaustion, may help prevent renal flares. This may perhaps be achieved by upregulating inhibitory receptor expression or blocking co-stimulation of CD4 T cells to maintain the exhausted phenotype.[6]

The primary objective of this pilot study was to explore the KAT2B expression on CD4+ T cells in patients with juvenile onset SLE and its relation to biomarkers of lupus flare and organ involvement. The secondary objective was to study the relationship between KAT2B expression on CD4+ T cells and SLE disease activity index (SLEDAI) and immunosuppressive therapy used. Our aim is to anticipate the role of epigenetics in lupus which might enhance targeted management of this disease and hence decrease the adverse effects of non-specific immunosuppressive therapy

METHODS

The study is a cross-sectional comparative study that was carried out at the Pediatric Allergy, Immunology and Rheumatology Unit, Children's Hospital, Ain Shams University. The patients' group comprised 30 subjects with pediatric SLE who fulfilled at least four of the SLICC classification criteria. Thirty age- and sex-matched healthy control children were recruited from the Outpatient Clinic, Children's Hospital, Ain Shams University after exclusion of any current infection or personal or family history of any autoimmune disorder.

A written informed consent was obtained from the patients' parents or legal guardian and the protocol gained approval of the Medical Research Ethics Committee of the Pediatric Department, Ain Shams University [Approval number: FMASU 3099/2017].

History taking included the age of presentation, presenting manifestation(s) and pattern of organ involvement such as renal, neuropsychiatric, and other symptoms e.g., heart and lungs. Medication history in terms of immunosuppressive therapy (steroids, azathioprine, mycophenolate mofetil and cyclophosphamide) was recorded. Results of renal biopsy and relevant neuroimaging and cardiopulmonary investigations were recorded as well.

Patients underwent physical examination in the form of general data including blood pressure measurement as well as cardiac, chest, abdominal and neurological examination to assess the SLE activity status and major organ involvement at recruitment.

Laboratory investigations. Complete blood counting (CBC) by Coulter LH 750 cell counter(Coulter, Electronics, Hialeah, FL, USA) with manual differential, erythrocyte sedimentation rate (ESR) by Westergren method, Anti-double stranded deoxyribonucleic acid (Anti-ds DNA) by enzyme-linked immune-sorbent assay (ELISA Quanta lite ds ELISA.www.nt-protons.com; negative <20 IU/ml), Complement-3 (C3) by nephelometry (MININEPH TM, The Binding Site Ltd, Birmingham, UK; reference range: 89-187 mg/dl). Serum creatinine and blood urea nitrogen (BUN), Complete urine analysis, twenty-four hours urinary protein results were also recorded. KAT2B expression on MNCs was estimated using Colorimetric Cell-Based ELISA Kit (CytoGlow™, Assay biotechnology, California, San Francisco, USA).

Assessment of SLE activity. Global SLE activity was measured using Systemic Lupus Erythematosus Disease Activity Index (SLEDAI).[8] SLEDAI was interpreted as follow: no activity equals 0, mild activity (1-5), moderate activity (6-10), severe activity (11-19) and very high severe activity ≥ 20.[9] Assessment of lupus-related renal activity was performed using the renal SLEDAI which is calculated by the four urinary parameters of SLEDAI. Scores for the renal SLEDAI range from 0 (inactive renal disease) to a maximum of 16. A SLEDAI score of ≥ 4 was taken as an indicator of active lupus nephritis.[10]

Statistical Analysis

The collected data was revised, coded, tabulated, and introduced to a PC utilizing the Statistical package for Social Science (SPSS 25, IBM). Data was presented and suitable analysis was done according to the type of data obtained for each parameter.

Descriptive statistics: was performed by mean, standard deviation (± SD) and range for parametric numerical data. Frequency and percentage of non-numerical data. Analytical statistics: was done using Student T, Correlation analysis (using Pearson's method and spearman's rho). The ROC Curve (Receiver Operating Characteristic) provides a useful way to evaluate the Sensitivity and specificity for quantitative Diagnostic measures. Probability (p) values < 0.05 were considered significant.

RESULTS

The studied population (30 patients) included 23 females (76.67%) and 7 males (23.33%); their ages ranged between 9 and 18 years with a mean (SD) of 15 (2.61) years. Major organ involvement was found in 22 patients (73%) of whom 18 (60%) had isolated lupus nephritis (LN), one had CNS lupus and LN, two had resolving carditis, and one had interstitial lung disease (table 1).

At enrollment, leucopenia was observed in 6 patients (20%) while isolated lymphopenia was detected in three (10%). In addition, twelve patients (40%) had anemia and 2 patients (7%) had thrombocytopenia. Anti-dsDNA was positive in 24 patients (80%) and C3 was consumed in 9 patients (30%). Proteinuria was detected in 67% of enrolled patients where 6 patients (20%) had mild proteinuria, 10 patients (33%) had moderate proteinuria and 4 patients (13%) had heavy proteinuria, among whom, one patient had RBCS casts.

The level of SLE activity among the studied patients using the SLEDAI revealed that 21 patients (70%) had mild activity with a mean SLEDAI score of 2, 8 patients (27%) had moderate activity with a mean score of 7, one patient suffered of severe activity with a score of 18.

All the studied patients (100%) were on corticosteroid therapy. Nineteen patients were receiving additional immunosuppressive drugs in the form of mycophenolate mofetil in 11 patients (37%), cyclophosphamide in six (20%), azathioprine in two (7%), and rituximab in only one patient.

KAT2B expression on MNCs was significantly lower in the patients' group than the control values (table 2). At a level of 1.077 ng/ml, KAT2B expression on MNCs was found to be a reliable discriminator between pSLE and normal status, with AUC of 0.807. The levels of sensitivity and specificity were 93.3% and 73.3%, respectively (Figure 1). Values of KAT2B expression on MNCs were comparable among males and females in both patients' and control groups and did not correlate significantly with their ages. It was significantly lower among patients with moderate and severe lupus activity as compared to patients with mild activity as assessed by the SLEDAI. (Table 3 and Figure 2)

The KAT2B expression on MNCs did not vary significantly according to the clinical or laboratory evidence of lupus nephritis (LN); p=0.67. It also was comparable between patients with proliferative LN (class III and IV LN) and non-proliferative LN (class II and V LN); p=0.65. However, there was significant negative correlation between 24-hour urinary protein levels and KAT2B expression on MNCs among patients with LN (p=0.024). No other significant correlations could be elicited other laboratory data of the SLE patients (Table 4).

Patients consuming add-on immunosuppressives seemed to have lower levels of KAT2B expression on MNCs than those who received only corticosteroids, but the difference did not reach statistical significance (p=0.086). Moreover, patients treated with cyclophosphamide were comparable to those treated with mycophenolate mofetil as far as the KAT2B expression on MNCs is concerned (p=0.968).

Table 1. Demographic and disease characteristics of the studied SLE patients.

Variable	N (%)	Mean (range)	SD
Females	23 (70%)		
Males	7 (30%)		
Age at enrollment (years)		15 (9-18)	2.61
Age at onset of SLE (years)		12.57 (7 – 15)	2.11
Duration in years		12.6 (0.08 - 6)	2.1
SLE with major organ affection	22 (73%)		
SLE with lupus nephritis (LN)	18 (63%)		
Proliferative LN class III & IV	11 (58%)		
Non-proliferative LN class II & V	8 (42%)		
Lupus nephritis and CNS lupus	1 (3%)		
Carditis	2 (6%)		
Interstitial lung disease	1 (3%)		
SLE without major organ affection	8 (24%)		

CNS: central nervous system, LN: Lupus nephritis; SLE: Systemic lupus erythematosus

Table 2. KAT2B expression on MNCs among SLE patients and healthy controls

Variable	Groups		Student t-test	
	Controls	Patients		
	Mean ± SD	Mean ± SD	t	p-Value
KAT2B expression on MNCs (ng/ml)	0.96±1.61	0.24±0.71	4.958	<0.001

KAT2B: Lysine acetyltransferase 2B; MNCs: Mononuclear cells; SD: Standard deviation; SLE: Systemic lupus erythematosus

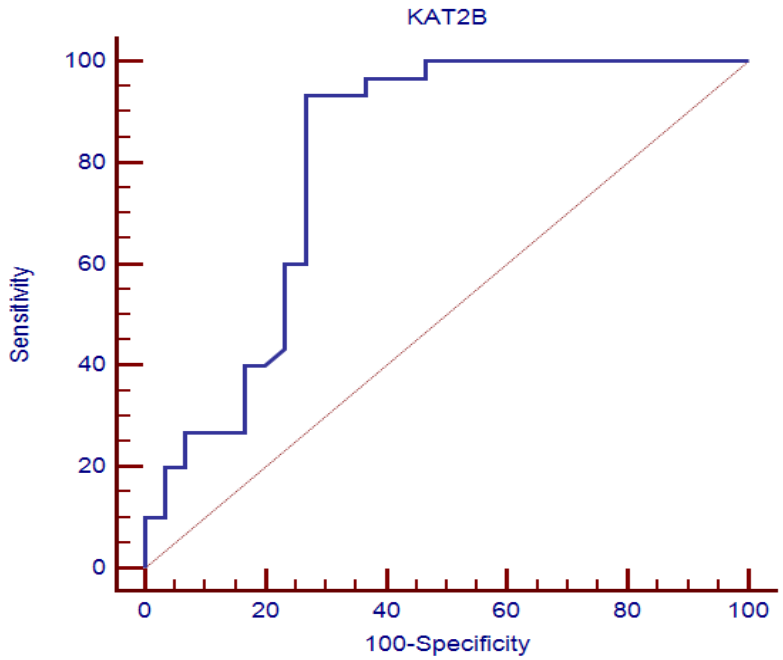

Figure 1. ROC curve analysis of the sensitivity and specificity of KAT2B expression in SLE
At the level of 1.077ng/ml, KAT2B expression on MNCs was found to be a reliable discriminator between lupus patients and healthy controls, with AUC 0.807 and 93.33% sensitivity and 73.33% specificity (p<0.001). AUC: Area under the ROC curve; KAT2B: Lysine acetyltransferase 2B; MNCs: Mononuclear cells; ROC: Receiver-operating characteristic; SLE: Systemic lupus erythematosus

Table 3. Variation of KAT2B expression on MNCs according to lupus activity as judged by SLEDAI

	SLEDAI		Student t-test	
	Mild N(%): 21 (70%)	Moderate - severe N(%):9 (30%)		
	Mean ± SD	Mean ± SD	t	p-Value
KAT2B expression on MNCs (ng/ml)	0.25±1.56	0.18±0.59	2.878	**0.03**

KAT2B: Lysine acetyltransferase 2B; N: Number; MNCs: Mononuclear cells; SD: Standard deviation; SLE: Systemic lupus erythematosus; SLEDAI: Systemic lupus erythematosus disease activity index; %: percentage.

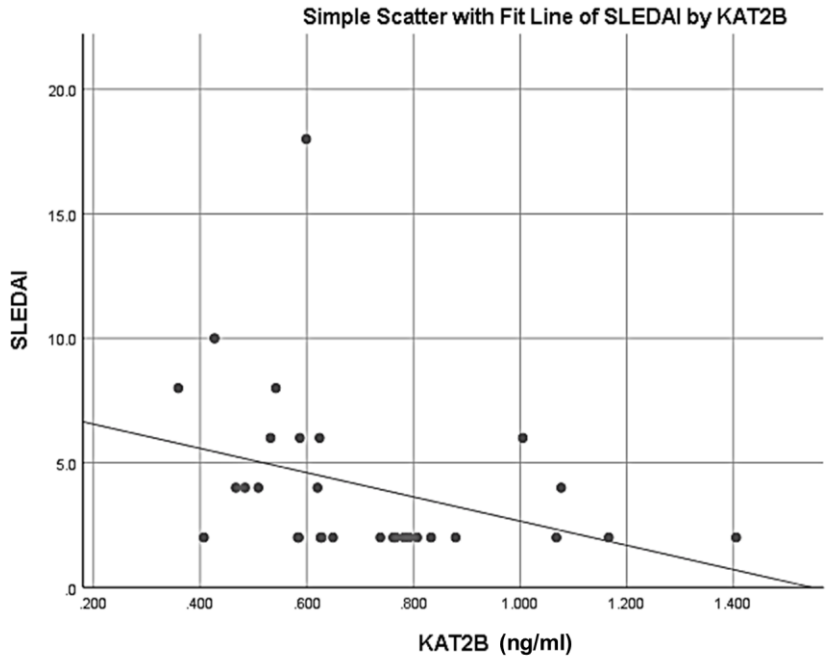

Figure 2. Negative correlation between SLEDAI scores and KAT2B expression on MNCs in the SLE patients (p=0.004).

KAT2B: Lysine acetyltransferase 2B; MNCs: Mononuclear cells; SLEDAI: Systemic lupus erythematosus disease activity index; SLE: Systemic lupus erythematosus

Table 4. Correlation between KAT2B expression on MNCs and some studied laboratory data

Test	KAT2B expression on MNCs	
	r	p value
ALC	0.030(P)	0.877
ESR	-0.157(P)	0.407
Anti-DNA	-0.139 (P)	0.465
C3	0.051(P)	0.788
24-hours urinary proteins	-0.41(S)	0.024*

* Significant; ALC: Absolute lymphocyte count; Anti-DNA: Anti-double stranded deoxyribonucleic acid; C3: complement 3; ESR: Erythrocyte sedimentation rate; KAT2B: Lysine acetyltransferase 2B; MNCs: Mononuclear cells; P: Pearson correlation; S: Spearman Correlation.

DISCUSSION

Lysine acetyltransferase 2B (KAT2B) affects gene transcription through DNA acetylation. There have been few conflicting studies about KAT2B expression and its role in the pathogenesis of autoimmune diseases and whether associated with flare or remission. In the current study, we found that KAT2B expression on MNCs was significantly lower among patients as compared to the healthy controls. Moreover, patients with moderate and severe lupus activity had significantly lower values than those with mild activity as judged by the SLEDAI.

In concordance with our findings, Forster et al[11] demonstrated that SLE-like disease was observed in mice with deficient KAT2B activity, concluding that acetyltransferase activity promotes self-tolerance of B lymphocytes and suppress autoimmune disease in mice. Likewise, a study on global histone H3/H4 acetylation in CD4+ T cells in 20 adult SLE patients versus 10 healthy controls noted that histone acetylation was significantly lower in patients with active disease (SLEDAI >5). These patients had decreased mRNA of acetylases including KAT2B and the hypoacetylation was directly proportional to the disease severity.[12] Again, KAT2B messenger RNA (mRNA) and protein were diminished in inflamed intestinal tissues of adult patients with inflammatory bowel disease as compared to normal ones studied. This

was attributed to down regulation of KAT2B which led to lower levels of interlukine-10 (IL10) that has anti-inflammatory effects.[13]

On the contrary, Zhou and co-workers[14] reported that acetylated histones H3 and H4 were significantly increased in adult patients with active lupus and their levels were correlated to disease severity. This finding was explained by the downregulation of histone deacetylases, HDAC2, HDAC7 that lead to increased acetylation with opening-up of the chromatin. Also, A study on the expression of microRNA 181, which has a role in autoimmunity, and KAT2B mRNA by reverse-transcriptase PCR, in 20 pediatric SLE patients and 9 controls revealed that microRNA 181 was significantly downregulated while KAT2B was upregulated in patients with active disease (SLEDAI $\geq$ 10) as compared to normal subjects. The authors suggested that microRNA 181 regulates KAT2B gene expression and by downregulation of this negative regulator KAT2B upregulation occurs. Also, upregulation of KAT2B has an impact on the ubiquitination of human double minute 2 homolog (Hdm2) and the release of tumor protein 53 (p53) which may lead to the induction of apoptosis.[15] A group of investigators found that KAT2B was upregulated and mediated the expression of inflammatory molecules promoting inflammation in mice exposed to renal toxins inducing nephritis and proteinuria.[16]

Almaani and coworkers[6] suggested that there is a protective role for KAT2B in SLE and that it may ameliorate or prevent renal flares through maintaining exhausted phenotype of CD8 T cells and its antiapoptotic effect. CD8 T-cell exhaustion was found to be negatively correlated with CD4+ T-cell co-stimulation and that it indicates a better prognosis of SLE and ANCA-associated vasculitis and KAT2B was considered a prognostic marker in the transcriptome of unseparated MNCs, where its level was found to be elevated in active disease.[17]

Therefore, based on the previous studies and ours, KAT2B seems to have differential effect in autoimmune diseases and its behavior is not uniform with respect to the pathogenesis of different autoimmune diseases. However, the triggering factor for KAT2B to perform as suppressor or exacerbator of autoimmunity still needs to be identified.

We found that KAT2B levels were inversely correlated with proteinuria and significantly decreased in patients with high levels of urinary proteins measured in 24-hour samples. This correlated well with our observation of low levels of KAT2B in active disease. However, no difference was found in KAT2B expression in patients with lupus nephritis (LN) compared to those without and among different classes of LN which may be related to its link to disease activity whether in the kidney or elsewhere. In other words, the expression is activity-linked whatever the organ affected is. Our observations are, however, limited by the sample size.

Our study has some limitations including the small sample size and the use of cell-based ELISA which is less accurate than the Western blot technique. The latter is not available in our country. Also, the cross-sectional design did not allow for accurately evaluating KAT2B expression in the remission state. The studied sample was not evenly distributed in terms of major organ involvement owing to the consecutive manner of enrollment and the limited sample size. This can be overcome by a stratified sampling manner in future studies.

From this pilot study, we may conclude that the low expression of KAT2B on MNCs could play a role in the pathogenesis of SLE in relation to disease activity pediatric SLE based on its lower expression in patients as compared to healthy controls and among patients with higher activity scores and higher levels of proteinuria.

Future wider scale studies are needed to evaluate the direct effect of KAT2B on the behavior of T and B cells in patients with pSLE and to validate our observation on whether KAT2B expression is related to a specific organ involvement in SLE or rather to disease activity whatever the organ affected is. A prospective study design is recommended to evaluate KAT2B levels during lupus activity and remission. This would give a clearer idea on its role in SLE control and hence potential KAT2B therapeutic targets.

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