

Evaluation of deoxyribonuclease 1-Like 3 as a potential regulator for immune activation in juvenile-onset systemic lupus erythematosus patients: a case-control study

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ABSTRACT

Objectives: This study aimed to evaluate the expression pattern of Deoxyribonuclease1-Like 3 (DNase1L3), an endonuclease that catalyses the degradation of chromatin within apoptotic or necrotic cells, at the mRNA and protein levels and their association with the disease activity and inflammatory markers in Juvenile-onset systemic lupus erythematosus (jSLE) patients.

Methods: 36 Juvenile-onset systemic lupus erythematosus patients and 25 healthy controls were included in the study. DNase1L3 and Interleukin (IL)-1 β expression in peripheral blood were determined using qRT-PCR, while ELISA was used to determine plasma levels of DNase1L3 and tumour necrosis factor (TNF)- α .

Results: DNase1L3 expression pattern at the mRNA and protein levels was significantly lower while IL-1 β and TNF- α levels were significantly higher in Juvenile-onset systemic lupus erythematosus patients than healthy controls. There were significant negative associations between DNase1L3 expression at mRNA and protein levels with SLEDAI-2K of patients. Also, DNase1L3 plasma levels of patients were inversely correlated with IL-1 β levels in a significant manner.

Conclusion: DNase1L3 could be involved in the immune regulation of jSLE patients and could be considered as a potential regulator of immune activation in those patients.

Keywords: DNase1L3, Juvenile-onset systemic lupus erythematosus (jSLE), inflammatory cytokines, IL-1 β , TNF- α .

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INTRODUCTION

Monogenic involvement (1) rather than heterogenous polygenic affection (2), has been shown to be among the major determinants in the development of paediatric-onset SLE, a peculiar cohort with distinct clinical and immunologic features (3,4). The French Genetic and Immunologic Abnormalities in SLE (GENIAL/LUMUGENE) study is a longitudinal cohort (64 patients) describing several laboratory and genetic aspects of juvenile-onset SLE (jSLE) patients (5). The investigators divide the cohort into three groups being a) syndromic SLE (10 patients), with those patients being characterized by distinct clinical characteristics including growth failure, intracranial calcifications, b) familial SLE (12 patients), whereby patients have either familial consanguinity or first-degree SLE relatives, and c) all other early-onset SLE (42 patients).

Hence, differences in the pathophysiologic pathways of monogenic involvement could lead to various presentations. Monogenic affection includes complement deficiencies, interferonopathies, and abnormalities in deoxyribonucleic acid (DNA) damage, clearance, and repair; with the latter being regulated by a group of nucleases including deoxyribonucleases (DNases) (6), which are a group of enzymes that catalyse the degradation of DNA molecules and thus prevent self-DNA recognition and subsequent damage (7). Four different DNases have been identified, including DNase I (8), DNase1L3 (DNase γ) (9), DNase II (10), and TREX1 (DNase III) (11-13). Similarly, distinct clinical phenotypes vary among different DNases, with clinical (9) and experimental (7) studies demonstrating that DNase1L3 deficiency is associated with an earlier SLE-onset, renal involvement, hypocomplementemia, and high anti-double stranded deoxyribonucleic acid antibodies titres (anti-ds DNA).

It is of note that a complex interplay between DNase1L3 and other aspects of the innate immunity has been demonstrated in several studies, including inflammasome activation and subsequent interleukin (IL)-1 β elevated levels (14), a proinflammatory cytokine that has been linked to lupus nephritis murine models (15). Moreover, several endonucleases (16) including DNase1L3 (17), have been linked to clearance of nuclear extra-cellular traps (NETs) that are strongly implicated in the pathogenesis of SLE (18). Impaired NETosis leads to a downstream of abnormal cytokine milieu including elevated tumour necrosis factor- α (TNF- α) (19), a cytokine that has been shown to be associated with several aspects of SLE (20).

In this case-control study, we aimed at determining DNase1L3 expression at mRNA and protein levels, IL-1 β and TNF- α levels, and their potential association with several aspects of the disease in a cohort of jSLE patients.

PATIENTS AND METHODS

Ethics

This study was approved by the ethics committee of National Research Centre, Giza, Egypt with an approval number 16/109 and has therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. All samples were obtained with the written informed consents of the subjects.

Study subjects

This study included 25 healthy subjects and 36 juvenile-onset SLE patients (age of onset ≤ 16 years) recruited from Rheumatology and Rehabilitation outpatient clinic, Kasr Al Ainy Hospital, Cairo University from March to July 2019. All patients fulfilled the 2012 Systemic Lupus International Collaborating Clinics (SLICC) classification criteria for SLE (21). Demographic and cumulative clinical manifestations were recorded, and disease activity at the last visit was assessed through the Systemic Lupus Erythematosus Disease Activity Index-2K (SLEDAI-2K) (22).

RNA extraction and quantitative real-time PCR

Total RNA was extracted and isolated from fresh blood of all subjects of the study populations using QIAamp RNA Blood Mini Kit (Qiagen, Germany) according to the manufacturer's instructions. For reverse transcription, RNA was reverse-transcribed to cDNA using HiSenScrip™ RH(-) cDNA Synthesis Kit (iNtRON Biotechnology, Korea) according to the manufacturer's instructions. Reverse transcription was performed under the following conditions: 5 min at 25°C, 45 min at 45°C and followed by 10 min at 85°C and the resulting cDNA was kept at -80°C until use.

A quantitative real-time PCR (qRT-PCR) was carried out to quantify the expression levels in triplicate of DNase1L3 and IL-1 β using TaqMan® DNase1L3 and IL-1 β Assay kits and TaqMan® Universal Master Mix (Applied Biosystems) using 7500 fast real-time PCR system according to the manufacturer's instructions. GAPDH was used as endogenous control to normalize the expression levels of DNase1L3 and IL-1 β . Relative quantification (Rq) was calculated using the $2^{-\Delta\Delta CT}$

threshold cycle method. Δ Ct was determined by subtracting the Ct values for GAPDH from the Ct values for the gene of interest. qRT-PCR was performed under the following conditions: 2 min at 50°C, 10 min at 95°C, followed by 45 cycles at 95°C for 15s and at 60°C for 1 min (23, 24).

Enzyme-linked immunosorbent assay (ELISA)

Plasma DNase1L3 and TNF- α levels of all study subjects were determined using Human DNase1L3 ELISA kit (Sunlong Biotech Co. Ltd, China) and TNF- α ELISA kit (Elabscience, Elabscience Biotechnology Co., Ltd), respectively according to the manufacturer's protocol.

Statistical analysis

Data were statistically analysed using SPSS version 19.0 software (SPSS Inc., Chicago, Illinois, USA). Non-parametric Mann-Whitney U test was used to compare the expression pattern of DNase1L3 and the inflammatory cytokine levels between groups. The correlation of DNase1L3 expression with the clinical data, SLEDAI score and the inflammatory cytokine levels of jSLE patients was done using Spearman correlation analysis. Data were presented as median and mean \pm SD. A P value of less than 0.05 was considered statistically significant.

RESULTS

Baseline characteristics

The study included 36 jSLE patients, of whom 35 were females (97%). The mean age of patients at onset and at time of sampling was 12.66 ± 3 and 24.5 ± 7 years, respectively, whereas the median disease duration (interquartile ratio) was 138 (115) months. Furthermore, 25 normal controls, 22 females (88%) and 3 males (12%), were involved with a mean age 26 ± 6.4 years. It is of note that 4/36 (11%) patients had a family history of SLE, and 4/36 (11%) had a family history of other autoimmune diseases. Cumulative clinical and serologic patients' characteristics, and the medications received at the time of sampling are shown in Table 1.

Expression pattern of DNase1L3 at mRNA and protein levels

DNase1L3 mRNA expression pattern was significantly reduced in our patients compared to the control group (Rq median = 0.37, $p = 0.001$), with a 2.7-fold decrease (Figure. 1a). Moreover, plasma levels of DNase1L3 were significantly lower among our patients compared to their controls ($p = 0.019$) (Figure. 1b).

Association of DNase1L3 with disease characteristics and serological data

Among the patients' recorded characteristics, there was no association of DNase1L3 expression and plasma levels with any of the different clinical and serological features (Data not shown). Interestingly, there was a significant negative correlation between DNase1L3 expression at mRNA ($r = -0.531$, $p = 0.001$) and protein levels ($r = -0.430$, $p = 0.012$) with disease activity (SLEDAI-2K). On the other hand, there was no association between DNase1L3 mRNA expression ($r = -0.222$, $p = 0.214$) and protein levels ($r = -0.111$, $p = 0.537$) with disease damage (SDI).

Expression levels of Interleukin (IL)-1 β and Tumour necrosis factor (TNF)- α

Interleukin-1 β expression pattern was significantly higher among our patients in comparison to the controls (Rq median = 43.8, $p = 0.001$) (Figure. 2a). Moreover, patients' TNF- α plasma levels were significantly higher than those detected among the controls ($p = 0.015$) (Figure. 2b).

Association of DNase1L3 levels with IL-1 β and TNF- α Apart from a weak negative correlation between patients' DNase1L3 protein and IL-1 β levels ($r = 0.3$; $p = 0.04$), there was no association between DNase1L3 mRNA expression and IL-1 β levels ($r = -0.1$; $p = 0.3$); and moreover, there was no association between DNase1L3 mRNA expression ($r = -0.13$; $p = 0.4$) and protein ($r = -0.2$; $p = 0.09$) levels with TNF- α levels.

Table 1. Baseline patients' characteristics*

	N = 36 (%)
Cumulative clinical manifestations	
Constitutional	28 (78)
Mucocutaneous	10 (28)
Neuropsychiatric	9 (25)
Nephritis	29 (80.5)
Arthritis	24 (67)
Serositis	10 (28)
Secondary antiphospholipid syndrome	16 (44.4)
Cumulative immune profile	
ANA	32(89)
Anti-ds DNA	29(80.5)
Hypocomplementemia	17 (47)
aPL	16(44.4)
Disease activity at sampling visit	
SLEDAI-2K (Median (IQR))	9 (7)
Disease damage at sampling visit	
SDI (Median (IQR))	1 (2)
Medications received at sampling visit	
Glucocorticoids	36(100)
Hydroxychloroquine	25(69.4)
Azathioprine	12(33.3)
Mycophenolate mofetil	6(16.7)
Cyclophosphamide	5(14)

*Unless indicated, data are presented in number (N) and percentage. Abbreviations: IQR: interquartile ratio; ANA: anti-nuclear antibodies; Anti-ds DNA: anti-double stranded deoxyribonucleic acid antibodies; aPL: antiphospholipid antibodies; SLEDAI-2K: Systemic Lupus Erythematosus Disease Activity Index-2K; SDI: Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index

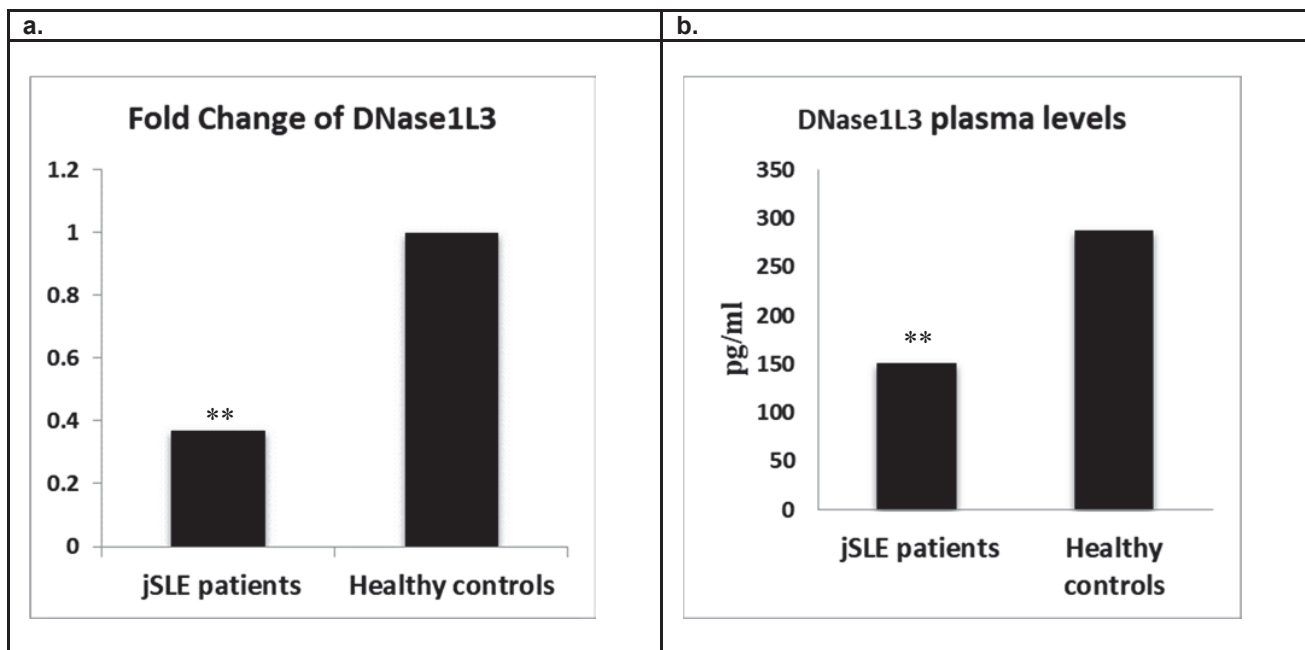


Figure. 1 a: Fold Change of DNase1L3 in jSLE patients relative to healthy controls. Data were presented as Rq median ($p = 0.001$, by Mann Whitney U Test).
b: Plasma levels of DNase1L3 in jSLE patients compared with healthy controls. Data were presented as median ($p = 0.019$, by Mann Whitney U Test).

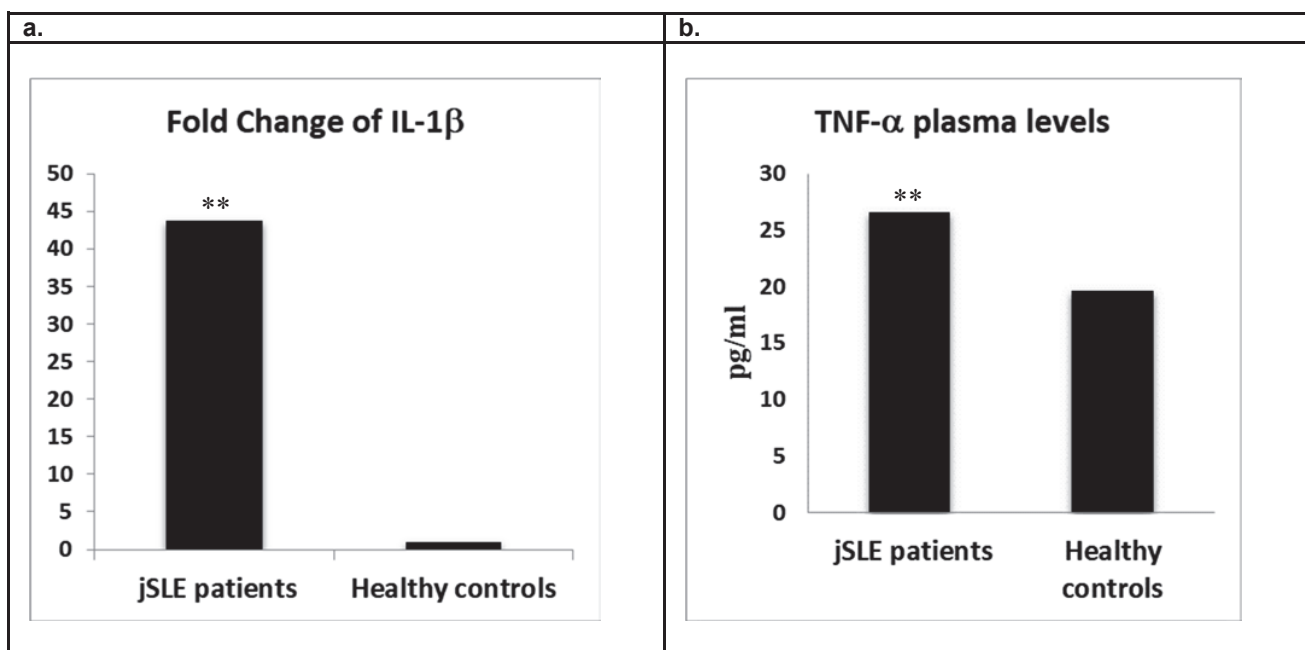


Figure. 2 a: Fold Change of IL-1β in jSLE patients relative to healthy controls. Data were presented as Rq median ($p = 0.001$, by Mann Whitney U Test).
b: TNF-α plasma levels in jSLE patients in comparison with healthy controls. Data were presented as median ($p = 0.015$, by Mann Whitney U Test)

DISCUSSION

DNase1L3 is one of the endonucleases that when aberrant, could lead to impaired DNA elimination and subsequent innate immunity activation and a downstream of altered cytokine milieu (14). Herein, we analyse the potential association of jSLE with DNase1L3 and the possible inter-play between DNase1L3 with IL-1β and TNF-α.

In our study, DNase1L3 pattern at both mRNA expression (2.7-fold) and plasma levels were significantly reduced in patients compared with the control group, hence resembling previous clinical study (25) and experimental studies (7). DNase1L3 LacZ/LacZ knockout (KO) murine models (7) that developed progressive anti-ds DNA levels increase at five weeks and was further followed by renal immune complex deposition and glomerulonephritis.

Interestingly, we found a significant negative correlation between DNase1L3 expression at mRNA and protein levels with disease activity (SLEDAI-2K). This is consistent with Zhao et al. (2017) who demonstrated a significant decrease in DNase1L3 level in the patients with active SLE versus those with inactive disease as well as in the patients with positive anti-ds DNA compared to those with negative anti-ds DNA (25).

We have investigated IL-1 β and TNF- α as potential markers associated with SLE and linked to DNase1L3. Although both cytokines' expression pattern was higher among our cases rather than the controls, TNF- α showed no association with DNase1L3 mRNA expression or protein level, whereas, apart from a weak negative correlation between patients' DNase1L3 protein and IL-1 β levels, there were no other significant associations between them. It is of note that several investigators demonstrated that DNase1L3 activity loss resulting in DNA accumulation could lead to subsequent toll like receptors (TLRs) activation (7,26) and hence, NF-kB dependent IL-1 β gene encoding transcription, a pro-IL-1 β protein (27,28). These insights into the pathophysiology entailing DNase deficiency give rise to potential therapeutic implications. Exogenous administration of recombinant DNase1L3 protein could prevent immunogenic DNA-dependent TLR signalling (29). Other proposed therapeutic implications include NET remnant elimination by DNase1 and 1L3 (17). In interest of SLE per se, recombinant DNase1 improved laboratory and renal markers in a lupus prone murine model (30), but these findings were not replicated when survival was set as a primary endpoint (31). Interestingly, DNase1L3, being an extracellular nuclease rather than an intracellular one, could lead to engineering and modification of DNase1L3 protein to enhance its nuclease activity and increase its half-life in circulation (7,29,32).

The main limitation of our study resides in the limited number of patients. However, our study strengths include investigate DNase1L3 in an Egyptian cohort, which is to the best of our knowledge was yet to be investigated, and we have studied its potential association with IL-1 β and TNF- α .

To conclude, our jSLE cohort demonstrated that DNase1L3 expression pattern at both mRNA and protein levels were significantly lower than healthy controls. Moreover, DNase1L3 expression at mRNA and protein levels inversely correlated with disease activity. On the other hand, IL-1 β and TNF- α levels were significantly elevated in jSLE patients in comparison to the control group; yet their association with DNase1L3 was unremarkable.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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