Int J Clin Exp Med 2021;14(12):2636-2640
www.ijcem.com /ISSN:1940-5901/IJCEM0136555

Original Article

Risk related single nucleotide polymorphisms in mitochondrial D-loops promotes the levels of reactive oxygen species in systemic lupus erythematosus

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Received June 20, 2021; Accepted October 27, 2021; Epub December 15, 2021; Published December 30, 2021

Abstract: Objective: Single nucleotide polymorphisms (SNPs) in the displacement-loop (D-loop) area of mitochondrial deoxyribonucleic acid (mtDNA) were found to be associated with an increased risk of systemic lupus erythematosus (SLE) in our previous research. This study mainly focused on the correlation of SLE risk-associated SNPs in the D-loops and the oxidative stress status. Methods: In total, 81 female Chinese patients diagnosed with SLE and 102 age-matched female controls were involved in this research from May 2017 to October 2017. The oxidative stress status was confirmed by measuring the reactive oxygen species (ROS) levels in the blood of SLE patients and controls. Student's t test was used to compare ROS levels between SLE patients and controls. Wilcoxon rank-sum test was used to analyze the correlation among SLE risk-associated SNPs, the clinical features and ROS levels. Results: The levels of ROS in SLE patients were remarkably higher than those in controls (655.025±49.748 vs. 533.284±30.033, *P*=0.030, 95% CI: 11.886, 231.595). In addition, the active SLE patients exhibited higher ROS generation compared with that of controls. Furthermore, the SLE susceptible SNP of 195T was linked with higher ROS generation (T: 41.823, C: 8.5, *P*=0.048). Conclusion: In summary, the SLE risk associated SNPs in the D-loop may initiate SLE by boosting oxidative stress levels.

Keywords: Systemic lupus erythematosus, mitochondrial DNA, displacement loop, ROS, oxidative stress

Introduction

Systemic lupus erythematosus (SLE) is a complex and heterogeneous autoimmune disease that damages various organs and systems such as mucous membranes, skin, serosal membrane, heart, kidneys, blood system and nervous system. The global prevalence of SLE is about 30-150 per 100,000, with an incidence ranging from 2.2-23.1 per 100,000 per year [1]. The etiology of SLE is involved in environmental and stochastic factors, as well as genetic susceptibility and other comprehensive factors with typical pathological features of multiple autoantibodies arising, but the mechanism of SLE remains unclear [2].

Mitochondrial deoxyribonucleic acid (mtDNA) is a relatively conserved round double-stranded DNA molecule that is composed of 37 genes with a size of 16 kB, and it is pivotal to maintain the stability of mitochondrial function [3]. Because of the abnormal reactive oxygen species (ROS) production, limited DNA-restoration capacity and the deficiency of protective histones, mtDNA is much more sensitive to DNA damage and much more easily acquires various mutations than nuclear DNA [4]. The D-loop region is composed of the leading-strand origin of replication and various important promoters for transcription, which are critical for mtDNA expression [5], thereby mutations and SNPs in this region can induce diseases by affecting the function of the mitochondria.

Endogenous ROS are mainly produced by respiratory burst of phagocytes, the mitochondrial electron transport chain, and lipid oxidation.

The abnormality of ROS levels has been proven to be a cause of oxidative damage to nearby or distant intracellular components, including DNA, lipids, proteins and phospholipids, so as to initiate subsequent cellular dysfunction or autoimmune responses [6]. Patients with SLE show higher ROS levels in body fluid compared with that of the normal population [7]. Elevated ROS levels are related to the release of cytochrome c, which may increase apoptosis and thereby accelerate the occurrence of SLE [8].

In previous studies, it was found that patients carrying 73G, 195T and 199C alleles were more susceptible to SLE [9]. In the current study, we measured the levels of ROS in SLE samples so as to analyze the relationship between these SNPs and the oxidative stress status.

Materials and methods

Specimen collection

Blood samples of 81 female Chinese patients with SLE were collected from the Second Hospital of Hebei Medical University between May 2017 and October 2017. All the patients were confirmed to have SLE based on the 1997 classification criteria by the American College of Rheumatology [10]. Patients who had other chronic inflammatory diseases were excluded from the study. The collected data of the SLE patients included age, sex, SLE disease activity index (SLEDAI), and clinical features such as anti-dsDNA, rash, ulcer, serositis, arthritis, lupus nephritis, lupus encephalopathy, and hematological abnormalities. Disease activity was assessed by SLEDAI. We defined the SLEDAI scores ≥6 as active, while those with <6 were stable. Simultaneously, 102 age-matched female controls who had no history of autoimmune diseases were included in the study. All procedures were in accordance with the Declaration of Helsinki and the study was supervised and approved of by the Ethics Committee of the Second Hospital of Hebei Medical University (2017-P031). Informed consent was signed by all the patients prior to enrollment.

Measurement of ROS

We collected peripheral blood samples from SLE patients and controls and measured the ROS levels using BBOxiProbe® Plasma Active Oxygen Detection Kit (BestBio Technology, Shanghai, China), following the manufacturer's instructions. In brief, 100 μL plasma was incubated with 10 μL 012 probes in a dark environment at 37°C for 30 minutes. The levels of ROS were analyzed by a Fluorescence Microplate Reader (BIOTEK, Winooski, VT, USA) with an excitation wavelength of 488 nm and an emission wavelength of 520 nm.

Statistical analysis

The relationship of the clinical features and risk-associated SNPs among the SLE patients was identified by the Chi-square test or Fisher's exact test. The Student's t test was used to compare ROS levels between SLE patients and controls. Wilcoxon rank-sum test was used to analyze the relationship between SLE risk-associated SNPs as well as the clinical features and ROS levels. SPSS 19.0 software (SPSS Inc., Chicago, IL, USA) was used for analysis of the data and statistics. Differences were regarded as statistically significant, when the *p* value was less than 0.05.

Results

In total, 81 female Chinese SLE patients (mean age 41.68±15.65 years) and 102 age-matched female controls (mean age 43.72± 16.11 years) were involved in the study. The clinical features of these patients are presented in **Table 1**. It was found that no difference was observed in clinical characteristics of these risk-associated SNPs by Chi-square test or Fisher's exact test. As shown in **Figure 1**, the levels of ROS in SLE patients were remarkably higher than that in healthy controls (655.025±49.748 vs. 533.284±30.033, *P*= 0.030, 95% CI: 11.886, 231.595). We divided these SLE patients into two groups as active and stable patients based on the SLEDAI score (**Table 2**), the active SLE patients exhibited higher ROS generation compared with that of stable patients (44.52, 31.57, *P*=0.028).

The potential relationship between ROS levels and SLE risk-associated SNPs including 73G/A, 195T/C, 199T/C were also evaluated by Wilcoxon rank sum test subsequently, as shown in **Table 3**, the 195 allele was identified to be associated with ROS levels, with the SLE susceptible T allele promoting the ROS generation

Table 1. Clinical characteristics of SLE patients

Clinicopathological Features			73*		p	195		p	197		p
			G	A		T	C		T	C	
		79	79	0	-	79	2		73	8	
Age	≤60	67	65	0	-	66	1	0.318	60	7	1
	>60	14	14	0		13	1		13	1	
SLEDAI	≥6 (active)	22	21	0	-	22	0	1	20	2	1
	<6 (stable)	59	58	0		57	2		53	6	
Anti-dsDNA	+	38	37	0	-	37	1	1	33	5	0.464
	-	43	42	0		42	1		40	3	
Rash	+	47	47	0	-	46	1	0.516	41	6	0.457
	-	34	32	0		33	1		32	2	
Ulcer	+	7	7	0	-	7	0	1	6	1	0.531
	-	74	72	0		72	2		67	7	
Serositis	+	8	8	0	-	8	0	1	8	0	1
	-	73	71	0		71	2		65	8	
Arthritis	+	29	29	0	-	29	0	0.535	26	3	1
	-	52	50	0		50	2		47	5	
Lupus	+	33	32	0	-	32	1	1	32	1	0.133
nephritis	-	48	47	0		47	1		41	7	
Lupus	+	15	15	0	-	15	0	1	15	0	0.340
encephalopathy	-	66	64	0		64	2		58	8	
Hematological	+	49	48	0	-	47	2	0.516	46	3	0.253
abnormalities	-	32	31	0		32	0		27	5	

SLE: Systemic lupus erythematosus; SLEDAI: SLE disease activity index; dsDNA: double-stranded deoxyribonucleic acid. *Seventy-nine SLE patients carry homozygous G/G at nucleotide 73, while two patients carry C/C at this nucleotide position.

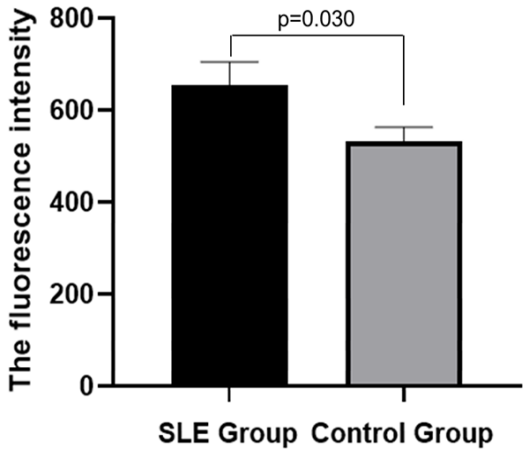

Figure 1. Reactive oxygen species level of groups. SLE: Systemic lupus erythematosus.

(T: 41.823, C: 8.5, *P*=0.048). These data demonstrated the SLE associated SNP in the mitochondrial D-loop can promote the ROS generation.

Discussion

An increasing amount of evidence supports the observation that mitochondrial disorders and abnormal oxygen metabolism play a rather significant role in the formation of SLE [11]. The SLE susceptible D-loop SNP of 195T was correlated with higher ROS levels in SLE patients. Our data implied that the SLE risk associated SNP might promote the ROS generation that initiates the SLE process.

The mtDNA D-loop is situated in the non-coding region, an area defined as a control region containing a promoter for RNA transcription and DNA replication [12]. We hypothesize that SNPs in this area may act on the electron transport chain to increase ROS production, which will generate auto-antigens, change mtDNA replication rate and disrupt the nuclear genome function thereby initiating the illness process [13, 14]. We have proven that the SLE susceptible 195T allele can promote ROS generation in SLE patients. The allele at position 195 lies in the hypervariable segment 2 (HV2), which has been found to be highly related to the cause of SLE, colon cancer and Alzheimer's disease [9, 15, 16].

Mitochondria are known as the powerhouses of the cell, producing most of the endogenous ROS and regulating programmed cell death [3]. ROS can modify cellular components and metabolites as well as increase tissue damage and cardiovascular events through lipoprotein-mediated pathways and oxidative stress [17, 18]. Consistent with the results of previous studies [7], we also found that SLE patients exhibit a high state of ROS when compared with that of healthy controls. Meanwhile, the increased ROS levels are positively correlated with SLEDAI scores. All of these imply that ROS might be involved in the SLE process as well as modify the degree of SLE. Oxidized lipoproteins

Table 2. Correlation between ROS and clinical characteristics of SLE

Clinicopathological Features		ROS	
		Mean Rank	*p*
Age	≤60	42.69	0.158
	>60	32.93	
SLEDAI	≥6 (active)	44.52	0.028
	<6 (stable)	31.57	
Anti-dsDNA	+	45.75	0.088
	-	36.8	
Rash	+	40.59	0.852
	-	41.57	
Ulcer	+	30.86	0.233
	-	41.96	
Serositis	+	41	1
	-	41	
Arthritis	+	40.4	0.863
	-	41.34	
Lupus nephritis	+	47.65	0.062
	-	36.56	
Lupus encephalopathy	+	48.1	0.195
	-	39.39	
Hematological abnormalities	+	43.13	0.313
	-	37.73	

SLE: Systemic lupus erythematosus; SLEDAI: SLE disease activity index; dsDNA: double-stranded deoxyribonucleic acid.

Table 3. Correlation between ROS and SLE risk-associated SNPs

SNP	Allele	Mean Rank	*p*
73*	G	-	-
	A	-	-
195	T	41.823	0.048
	C	8.5	
199	T	40.589	0.635
	C	44.75	

SLE: Systemic lupus erythematosus; SNP: single nucleotide polymorphisms; ROS: Reactive oxygen species; *Seventy-nine SLE patients carry homozygous G/G at nucleotide 73, while two patients carry C/C at this nucleotide position.

are a marker of lipid peroxidation-derived aldehydes (LPDA) that can change the protein immunogenicity in SLE so as to modify SLEDAI scores [17, 18]. All of these support lipid peroxidation to play a crucial role not only in the pathogenesis of SLE but also in its progression. ROS-modified DNA produces new antigens epitopes, which combine with circulating anti-DNA antibodies to form immune complexes that are deposited in different tissues and organs so as to initiate the pathogenesis of SLE [13].

In summary, the SLE-risk associated D-loop SNPs may be able to cause SLE by boosting the oxidative stress level.

Disclosure of conflict of interest

None.

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