Serum lipoxin A4 as a biomarker for systemic lupus erythematosus

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Introduction
Systemic lupus erythematosus (SLE) is an immune-complex mediated autoimmune disease characterized by protean clinical manifestations and fluctuating disease course. It was proposed that progression and flares of lupus and lupus nephritis are due to decreased production of lipoxin A4 (LXA4) and enhanced production of leukotrienes by the renal tissue and/or infiltrating leukocytes and macrophages.

Objective
The aim of this study was to assess the levels of serum LXA4 in SLE patients and in healthy controls, and to correlate them with various clinical and laboratory data as well as renal biopsy and disease activity indices.

Patients and methods
Forty adult female SLE patients were included in this study. The SLE patients were divided into two groups: group I included 20 patients without nephritis and group II included 20 patients with nephritis. Forty apparently healthy age-matched women served as the control group. Patients and controls were assessed for serum LXA4 using enzyme linked immunosorbent assay. Disease activity was assessed using systemic lupus erythematosus desease activity index (SLEDAI) and renal SLEDAI. Renal biopsy was performed for patients with lupus nephritis.

Results
SLE patients showed a higher median level of serum LXA4 compared with the control group (0.24 vs. 0.15 ng/ml), but with no significant statistical difference ($P = 0.097$). Moreover, there was no statistically significant difference in serum LXA4 between SLE patients with and without nephritis and the control group ($P = 0.142$). There was no significant correlation between serum LXA4 and various clinical and laboratory data, as well as renal biopsy and disease activity index.

Conclusion
LXA4 was suggested to be an important biomarker to search for in SLE. Our study showed no significant statistical difference between SLE patients and the control group as regards serum LXA4. Assessment of urinary LXA4 is recommended as it may be more valuable than serum LXA4 in reflecting organ affection in SLE, as well as disease activity, and comparing it with serum LXA4 level.

Keywords:
activity scores, lupus nephritis, serum lipoxin A4, systemic lupus erythematosus

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Introduction

Systemic lupus erythematosus (SLE) is an immune-complex mediated autoimmune disease characterized by protean clinical manifestations and fluctuating disease course. The exact pathoetiology of SLE is not fully understood, but it is believed to be multifactorial, with environmental, neuroendocrine, genetic, hormonal, and infectious factors playing a role [1].

Lupus nephritis (LN) is the most serious organ manifestation of SLE. It occurs in a relevant proportion of SLE patients and if not recognized and treated in time, it may lead to renal failure and death [2]. The medical therapy for LN depends on the severity of the disease. Thus, finding reliable biomarkers for LN will help in evaluating disease activity, identifying patients at risk for kidney damage, and facilitating early diagnosis and intervention to improve favorable outcomes [3].

Lipoxin A4 (LXA4), a lipoxygenase-derived eicosanoid, is anti-inflammatory and was one of the first proresolving mediators identified, as its appearance signals the resolution of acute inflammation. LXA4 has the specific proresolution actions of limiting PMN recruitment and adhesion. It essentially serves as braking signals for PMN-mediated tissue injury [4].

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Continued inflammation seen in lupus could be due to failure of the resolution of inflammation. Thus, the balance between inflammation and resolution is disturbed more in favor of proinflammatory events and/or failure of resolution, thereby inducing molecules to be produced leading to nonresolution of inflammation. This leads to delay in the healing/repair process and so tissue/organ damage continues [5].

Objective

The aim of this study was to assess the levels of serum LXA4 in SLE patients and healthy controls and to correlate them with various clinical and laboratory data, as well as renal biopsy and disease activity indices.

Patients and methods

Patients

Forty adult female SLE patients, attending the outpatient clinic or admitted in the inpatient unit of the Rheumatology and Rehabilitation Department, Faculty of Medicine, Cairo University Hospitals, were included in this work. They were diagnosed according to the American College of Rheumatology revised criteria for SLE [6]. Their ages ranged from 18 to 40 years and disease duration ranged from 1 to 20 years. Forty healthy age-matched women served as the control group. Patients with a diagnosis of overlap syndrome (coexistence of lupus with other connective tissue diseases such as rheumatoid arthritis or scleroderma), and with conditions that may affect the serum level of LXA4, such as diabetes mellitus, inflammatory lung diseases (e.g. bronchial asthma), and coronary heart disease, were excluded from this study.

We followed our committee's ethical guidelines in Cairo University, and informed consent was obtained from all participants according to the Declaration of Helsinki, General Assembly, December 2014.

All patients were subjected to full history taking, clinical examination, assessment of disease activity using SLEDAI [7] and renal SLEDAI [8], laboratory investigations (including ESR, CBC, liver function tests, kidney function tests, complete urine analysis, 24 h urinary proteins, ANA, anti-ds DNA, and C3 and C4), and assessment of serum LXA4. Patients with established LN (persistent hematuria, proteinuria, and casts) underwent renal biopsy. The renal pathology was classified according to the revised ISN/RPS system [9].

Assessment of serum lipoxin A4

Blood samples were obtained from all patients and controls. Sera were separated by centrifugation and stored at −20°C for later analysis of LXA4 level using enzyme linked immunosorbent assay (ELISA). LXA4 in serum was quantified using an ELISA kit with Product No.: EA45 (purchased from Oxford Biomedical Research Inc., USA). LXA4 was extracted from serum before analysis using C18 Sep-Pak Light Column (#23501; Waters Corporation) according to the manufacturer's instructions.

Statistical analysis

Statistical analysis was carried out using SPSS computer software package, version 15.0 (2006; Echosoft Corporation, USA). Qualitative data were expressed as frequencies and percentages. Quantitative data were expressed as mean ± SD for parametric data and median (25th–75th percentiles) for nonparametric data. Differences between groups were assessed using the one-way analysis of variance and Kruskal–Wallis test for parametric data and nonparametric data, respectively. If significant, appropriate post-hoc multiple comparison and Mann–Whitney tests were applied to identify exactly where the differences were. Correlation analysis between variables was carried out by applying Pearson's ranked correlation test and Spearman's ranked correlation test. All tests were two tailed, and a P value less than 0.05 was considered significant.

Results

The ages of the 40 adult SLE patients ranged from 18 to 50 years, with a mean age of 29.55 ± 7.7, and the disease duration ranged from 1 to 20 years, with a mean of 6.99 ± 4.72. Forty healthy age-matched women served as the control group, and their ages ranged from 18 to 50 years, with a mean of 32.9 ± 10.35 (Tables 1 and 2). The SLE patients were divided into two groups:

(1) Group I included 20 SLE patients without nephritis.
(2) Group II included 20 SLE patients with nephritis, defined by the renal parameter of the SLEDAI score as those patients having a renal SLEDAI of at least 8 (≥2 abnormal results for renal parameters on at least two occasions).

Renal biopsy of the 20 SLE patients with nephritis showed that four (20%) patients had LN class II, six (30%) had class III, seven (35%) had class IV, and three (15%) had class V.

SLE patients showed a higher median level of serum LXA4 compared with the control group (0.24 vs. 0.15 ng/ml), but with no significant statistical difference (P = 0.097) (Table 3).

Moreover, there was no statistically significant difference in serum LXA4 between SLE patients with and without nephritis and the control group (P = 0.142) (Table 4).

Table 1 Demographic data of group I and group II systemic lupus erythematosus patients

Variables	Group I (*n* = 20)	Group II (*n* = 20)
Age (years)		
Range	18–40	19–50
Mean ± SD	27.9 ± 5.65	31.2 ± 9.17
Disease duration (years)		
Range	1–20	1–13
Mean ± SD	8.05 ± 5.42	5.93 ± 3.73

Table 2 Clinical data of group I and group II systemic lupus erythematosus patients

Variables	Group I (*n* = 20) [*n* (%)]	Group II (*n* = 20) [*n* (%)]
Oral ulcers	13 (65)	11 (55)
Malar rash	12 (60)	15 (75)
Discoid rash	1 (5)	2 (10)
Photosensitivity	11 (55)	10 (50)
Alopecia	6 (30)	4 (20)
Arthritis	13 (65)	11 (55)
Myositis	0	1 (5)
Cardiovascular manifestations	8 (40)	12 (60)
Hypertension	1 (5)	7 (35)
Pulmonary manifestations	14 (70)	12 (60)
Pulmonary hypertension	2 (10)	4 (20)
Neuropsychiatric manifestations	6 (30)	5 (25)
Vasculitic lesions	5 (25)	6 (30)

Table 3 Comparison between the 40 systemic lupus erythematosus patients and the control group as regards serum lipoxin A4

Variables	SLE patients (*n* = 40)	Controls (*n* = 40)	*P* value
Serum LXA4 (ng/ml)			
Range	0.013–2.5	0.02–0.24	0.097
Median	0.24	0.15	
25th–75th percentile	0.1–0.3	0.1–0.21	

LXA4, lipoxin A4.

Table 4 Comparison between the median levels of serum lipoxin A4 in group I, group II, and the control group

Variables	Group I (*n* = 20)	Group II (*n* = 20)	Control (*n* = 40)	*P* value
Serum LXA4 (ng/ml)				
Range	0.02–0.4	0.013–2.5	0.02–0.24	0.142
Median	0.19	0.24	0.15	
25th–75th percentile	0.07–0.25	0.06–0.4	0.1–0.215	

LXA4, lipoxin A4.

There was no statistically significant difference between SLE patients with clinical parameters and those without various clinical parameters as regards serum LXA4 (Table 5).

No significant correlation was found between the level of serum LXA4 and laboratory data of the SLE patients (Table 6).

Moreover, there was no statistically significant difference between the levels of serum LXA4 in SLE patients with positive or negative anti-DNA antibodies (P = 0.987) and in SLE patients with normal or consumed C3 (P = 0.68) and C4 (P = 0.83) (Tables 7 and 8).

There was no significant correlation between the level of serum LXA4 and SLEDAI (r = 0.136, P = 0.417) and renal SLEDAI (r = 0.197, P = 0.235) of all SLE patients, as well as the activity scores of the two SLE groups (Table 9).

Moreover, no significant statistical difference was found between serum LXA4 levels within the WHO classes of LN in SLE patients with nephritis (P = 0.25) (Table 10).

Discussion

Eicosanoids form one of the most complex networks in the body controlling many physiological and pathophysiological processes, including inflammation, autoimmunity, and cancer. Persisting eicosanoid pathways are thought to be involved in the development of rheumatic diseases, and targeting this pathway might enable improved treatment strategies [10].

Das [5] proposed that the progression and flares of lupus and LN are due to decreased production of LXA4 and enhanced production of leukotrienes (LTs) by the renal tissue and/or infiltrating leukocytes and macrophages.

This was a novel study performed to assess the levels of serum LXA4 in SLE patients and in healthy controls and to correlate them with various clinical and laboratory data, as well as renal biopsy and disease activity indices.

SLE patients showed a higher median level of serum LXA4 compared with the control group (0.24 vs. 0.15 ng/ml), but with no significant statistical difference (P = 0.097).

A study conducted by Wu *et al.* [11] for detecting the temporal changes in blood and urinary LXA4, leukotriene B4 (LTB4), and urinary leukotriene E4 (LTE4) in 49 children with Henoch–Schönleinpurpura showed that blood and urinary LXA4 during the active phase (before treatment) was higher than that in controls and further increased in early resolution. This was contrary to the blood LTB4 and urinary LTB4 and LTE4, which showed an early increase in the active phase and a subsequent decrease during early resolution. These temporal changes between gradually enhanced LXA4 production and gradually suppressed

Table 5 Comparison between systemic lupus erythematosus patients with clinical manifestations and systemic lupus erythematosus patients without various clinical manifestations as regards serum lipoxin A4

Variables	Serum LXA4 (ng/ml)Median (25th–75th percentile)		*P* value
	Patients with	Patients without	
Mucocutaneous manifestations	0.23 (0.11–0.27)	0.285 (0.02–0.4)	0.71
Arthritis	0.18 (0.08–0.26)	0.25 (0.17–0.38)	0.24
Myositis	0.02	0.24 (0.12–0.31)	0.12
Cardiovascular manifestations	0.17 (0.04–0.3)	0.24 (0.18–0.38)	0.2
Pulmonary manifestations	0.24 (0.15–0.29)	0.19 (0.08–0.38)	0.88
Pulmonary hypertension	0.24 (0.09–0.34)	0.24 (0.09–0.31)	0.88
Neuropsychiatric manifestations	0.15 (0.06–0.29)	0.24 (0.15–0.36)	0.26
Vasculitic lesions	0.22 (0.1–0.25)	0.24 (0.11–0.38)	0.47

LXA4, lipoxin A4.

Table 6 Correlation of serum lipoxin A4 with laboratory data in all systemic lupus erythematosus patients

Variables	Serum LXA4	
	r	*P* value
ESR	0.165	0.323
Hb	–0.133	0.425
WBC	–0.119	0.476
Platelets	–0.043	0.797
ALT	0.085	0.613
AST	0.021	0.901
Albumin	–0.107	0.524
Creatinine	0.037	0.827
24 h protein in urine/day	0.153	0.359

ALT, alanine aminotransferase; AST, aspartate aminotransferase; LXA4, lipoxin A4; WBC, white blood cell.

LTB4 and LTE4 generation suggest eicosanoid class switching during acute inflammation and resolution.

In another study, Wu *et al*. [12] investigated the expression levels of LXA4, LTB4, and 15-lipoxygenase in children with acute poststreptococcal glomerulonephritis (APSGN). Blood and urinary levels of LXA4 and LTB4 were measured with ELISA within 3 days (acute phase), 10–14 days (early resolution phase), and 6–8 weeks (late resolution phase) after the onset of APSGN in 22 patients. Expression levels of both LXA4 and 15-LO in leukocytes and glomeruli were upregulated during the acute phase of disease, further peaking between days 10 and 14, and remained increased after 6–8 weeks of APSGN onset. In contrast, blood and urinary levels of LTB4, as well as the number of glomerular PMNs, peaked during the acute phase of disease and then decreased during the resolution phase.

In our study, there was no statistically significant difference in serum LXA4 between SLE patients with and without nephritis and the control group ($P = 0.142$).

Wu *et al*. [11] reported that the levels of blood and urinary LXA4 in patients with nephritis were lower than those in patients with purpura only in the early resolution of HSP. These data suggest that insufficiency of LXA4 in the human body, which weakens the anti-inflammatory and antifibrotic role, may be responsible for the patients with HSP with more serious illness.

The insignificant statistical difference between all SLE patients and the control group as regards serum LXA4, and also between SLE patients with and without nephritis and the control group could be explained by the fluctuating levels of LXA4 in blood as the blood values of LXA4 increase and decrease while the urine values remain elevated [13].

Moreover, there was no significant correlation between serum LXA4 and different laboratory parameters of the SLE patients.

In their study of the expression levels of LXA4,LTB4, and 15-lipoxygenase in children with APSGN, Wu *et al*. [12] reported that no significant correlations were found between the temporal changes of blood LXA4 and the glomerular filtration rate, and the degree of hypertension.

Our results also showed that there was no statistical significance in serum LXA4 levels within different WHO classes of LN in SLE patients with nephritis.

Wu *et al*. [14] studied the effect of LXA4 on TNF-alpha-induced production of interleukins and proliferation of rat mesangial cells. Cultured glomerular mesangial cells were treated with TNFa (10 ng/ml), with or without preincubation with LXA4, at different concentrations. They found that TNFa-stimulated proliferation, release of proteins, and expressions of mRNA of IL-1beta and IL-6 in mesangial cells were inhibited by LXA4 in a dose-dependent manner. The marked increments in mRNA expression and protein synthesis of cyclin E induced by TNF-alpha in parallel with proliferation of mesangial cells were downregulated by LXA4.

Table 7 Comparison between systemic lupus erythematosus patients with positive and negative anti-ds DNA as regards serum lipoxin A4

Variable	Anti-ds DNA		*P* value
	Positive (*n* = 29)	Negative (*n* = 11)	
Serum LXA4 (ng/ml) [median (25th–75th percentile)]	0.22 (0.1–0.32)	0.24 (0.08–0.26)	0.987

LXA4, lipoxin A4.

Table 8 Comparison between systemic lupus erythematosus patients with normal and consumed C3 and C4 as regards serum lipoxin A4

Variables	C3		*P* value	C4		*P* value
	Normal	Consumed		Normal	Consumed	
Serum LXA4 (ng/ml)						
Median (25th–75th percentile)	0.24 (0.1–0.28)	0.24 (0.08–0.4)	0.68	0.24 (0.07–0.34)	0.22 (0.11–0.29)	0.83

LXA4, lipoxin A4.

Table 9 Correlation of serum lipoxin A4 with activity scores in group I and group II systemic lupus erythematosus patients

Variables	Serum LXA4			
	Group I		Group II	
	R	*P* value	*r*	*P* value
SLEDAI	0.03	0.89	0.63	0.79
Renal SLEDAI	—	—	0.1	0.67

LXA4, lipoxin A4.

Table 10 Comparison between serum lipoxin A4 levels in WHO classes of lupus nephritis in group II systemic lupus erythematosus patients

WHO classes of LN	Serum LXA4 (ng/ml)	
	Median (25th–75th percentile)	*P* value
Class II	0.23 (0.1–0.37)	0.25
Class III	0.32 (0.13–0.46)	
Class IV	0.25 (0.24–0.5)	
Class V	0.1 (0.02–0.22)	

LN, lupus nephritis; LXA4, lipoxin A4.

Conclusion

LXA4 was suggested to be an important biomarker to search for in SLE. This was a novel study performed to assess the levels of serum LXA4 in SLE patients and to correlate them with various clinical and laboratory data. It showed that there was no significant statistical difference between all SLE patients and the control group as regards serum LXA4, and also between SLE patients with and without nephritis and the control group. Moreover, there was no significant statistical correlation with various clinical and laboratory data, as well as renal biopsy and disease activity index. We therefore recommend performing longitudinal studies with larger number of patients to follow changes in serum LXA4 levels before, during, and following SLE flares, and comparing their levels with other proinflammatory molecules such as LTs.

We also recommend assessment of urinary LXA4 as it may be more valuable than serum LXA4 in reflecting organ affection in SLE, as well as disease activity, and comparing it with serum LXA4 level.

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Conflicts of interest

There are no conflicts of interest.

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