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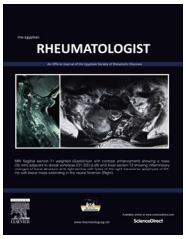

SHORT COMMUNICATION

The alternative complement pathway activity may depend on plasma malondialdehyde level in systemic lupus erythematosus patients: Preliminary results

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KEYWORDS

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Abstract *Background:* Malondialdehyde (MDA) is a marker of oxidative stress (OS) and one of the major alternative complement pathway (ACP) activators associated with systemic lupus erythematosus (SLE) activity. ACP is the principal mediator of SLE inflammation and progression.

Aim of the work: To investigate the association between the ACP functional activity and plasma MDA in SLE patients.

Patients and methods: Sixteen consecutive SLE patients were analyzed for their complement profile and oxidative stress measurement. 60 healthy subjects were included as a control group. The Complement components C3, C4 and properdin-factor B (PfB) were assessed, ACP activity was assayed according to alternative hemolytic 50 (AH_{50}). Plasma total lipid peroxide quantification was performed by assessing the plasma MDA. Total antioxidant capacity was measured with oxygen radical absorbance capacity (ORAC). OS ratio was calculated by dividing the total antioxidant capacity by MDA.

Results: Sixteen patients (13 females and 3 males) with a mean age of 27.86 ± 6.26 years and a disease duration 69.65 ± 54.65 months were included. There was a significant increase of MDA in the patients (MFI = 613 ± 56.21) compared to the control (MFI = 460 ± 37.85) ($p = 0.003$). C3 was significantly consumed and MDA increased in the low AH50 compared to the normal AH50 patients ($p = 0.02$ and $p = 0.01$ respectively). AH50 significantly negatively correlated with the C3 ($p = 0.02$) and MDA ($p = 0.048$). There was lack of any association between ORAC and ACP. Properdin factor B significantly negatively correlated with C3 ($p = 0.007$).

Conclusions: These initial results encourage future in-depth studies on the association of OS–ACP in SLE pathogenesis.

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1. Introduction

Systemic lupus erythematosus (SLE) is a multisystem autoimmune disorder characterized by microvascular inflammation and development of autoantibodies [1]. The exact cause of SLE is unknown; multiple factors including genetic and environmental [2] and various key players as cytokines [3,4] oxidative stress [5] and apoptosis [6] are implicated in the pathogenesis of the disease. Dysfunction of the T lymphocytes, B lymphocytes and dendritic cells, production of antinuclear autoantibodies and the loss of self-tolerance reveal the defective immune regulatory mechanisms [7] and increased oxidative damage [8,9].

The past decade has known intensive research efforts concerning the two major damage factors in SLE: oxidative stress (OS) and the alternative complement pathway (ACP) [10,11]. Complement was found to bind to malondialdehyde (MDA) epitopes and protects from oxidative stress [12]. Complement plays a dual role in the progression of SLE since it has important protective functions, such as the clearance of immune complexes and apoptotic cells, but is also a mediator of renal inflammation. However, selectively inhibiting the ACP is beneficial, presumably because of protective contributions from the classical and/or lectin pathways [13].

Oxidative stress contributes to immunomodulation which may lead to autoimmune diseases as SLE, antiphospholipid syndrome, rheumatoid arthritis (RA) [14–16], scleroderma [17] and Behcet's disease [18]. Reactive oxygen species are implicated in SLE and oxidative stress has the potential to elicit an autoimmune response, to contribute to the pathogenesis and could be useful when determining a prognosis [19]. Oxidative imbalance with an increase in MDA and a decrease in antioxidants plays a pathogenic role in the progression of SLE disease [20,21] and a possible cause of disease activity [22]. Inhibition of oxidative stress may represent newly discovered molecular and cellular targets for the treatment of SLE [7]. Antioxidants may protect against development of RA or SLE by combating oxidative stress [23,24].

Oxidative stress plays an important role in many aging diseases, including cardiovascular disease and age-related macular degeneration (AMD). Complement factor H risk allele confers higher complement activation and cell lysis activity in AMD by modulating oxidative stress and interacting with oxidized phospholipids [25]. However, these two lesional factors have not been analyzed simultaneously in this SLE.

The current study aimed to describe some evidence for OS–ACP association with a hope to initiate further studies to fill this gap in such a complex inflammatory disorder.

2. Patients and methods

This pilot study involved 16 consecutive SLE patients attending different wards of the Military University Hospital of Oran, Algeria fulfilling the 1997 American college of Rheumatology criteria [26]. Sixty healthy subjects (30 females and 30 males) of matched age served as a control. The study protocol was fully approved by the ethics committee of the Regional Military University Hospital of Oran (Oran, Algeria). All patients gave written informed consent. Other diseases such as diabetes mellitus, rheumatoid arthritis and antiphospholipid syndrome that may cause oxidative stress were excluded.

The plasma was separated and stored at −80 °C until analysis. The Complement components C3, C4 and properdin-factor B (PfB) were assessed in all samples by nephelometry laser (Image 800, Beckman Coulter, USA). ACP activity was assayed according to previously described method AH_{50} [27]. Plasma total lipid peroxide quantification was performed by malondialdehyde (MDA) level measurement using thiobarbituric acid reactive substance (TBARS) method and the results were given as mean fluorescence intensity (MFI) [28,29]. Total antioxidant capacity was measured with oxygen radical absorbance capacity (ORAC) with a 1:150 plasma dilution. The results were given as area under curve (AUC) [30]. OS ratio was calculated by dividing the total antioxidant capacity by MDA [31].

Statistical analysis was done by using SPSS (IBM software version 20.0 Chicago-USA). Student's *t*-test was performed for means comparison. In order to investigate whether or not OS parameters and plasma ACP activity were correlated, two tailed Pearson correlation was carried out. Significance was defined as $p \leqslant 0.05$.

3. Results

Sixteen patients (13 females and 3 males) with a mean age of 27.86 ± 6.26 years and a disease duration of 69.65 ± 54.65 months were included. The antinuclear antibody (ANA) positivity, disease activity and medications received of the active and inactive SLE patients are presented in Table 1. The 60 control (30 females and 30 males) had a mean age of 27.88 ± 8.28 years.

There was a significant increase of MDA in the patients (MFI = 613 ± 56.21) compared to the control (MFI = 460 ± 37.85) ($p = 0.003$). When SLE patients were divided into two groups according to their ACP activity AH_{50} (<80% and ⩾80%), both groups showed higher plasma MDA adducts than controls, but the levels were much greater in the low AH_{50} group. Interestingly, only C3 and MDA changed when the ACP was consumed (Table 2). The lack of any association between antioxidant defense capacity (ORAC) and ACP (Table 3) was unexpected.

The MDA level significantly correlated with AH_{50}, indicating that increased complement ACP activity parallels with lipid peroxidation endproduct levels (Table 3). Furthermore, the estimation curve by linear regression shows that MDA may explain ACP function by nearly 26% ($R^2 = 0.27$, $p = 0.048$) (Fig. 1).

Table 1 Characteristics of the active and inactive SLE patients.

Characteristic mean ± SD or *n* (%)	SLE patients (*n* = 16)	
	Inactive (*n* = 5)	Active (*n* = 11)
Age (years)	31.67 ± 7.2	24.1 ± 5.3
Disease duration (months)	95.3 ± 59.1	44 ± 50.2
SLEDAI	–	10 ± 2.3
ANA positivity	5 (100)	10
Prednisone intake	3 (60)	11
Chloroquine intake	4 (80)	9

SLEDAI: systemic lupus erythematosus disease activity index, ANA: antinuclear antibodies.

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Table 2 Complement factors and oxidative balance according to the alternative complement pathway activity in SLE patients.

Mean ± SD	SLE patients (n = 16)		
	AH_{50} N (n = 5)	AH_{50} low (n = 11)	p
PfB (mg/dl)	54.4 ± 13	18.1 ± 14.5	0.42
C3 (mg/dl)	93.7 ± 7.8	59.76 ± 7.9	0.02*
C4 (mg/dl)	8.1 ± 1.4	8.87 ± 0.5	0.54
ORAC (AUC)	8.7 ± 0.08	8.49 ± 0.11	0.21
MDA (MFI)	532.2 ± 40.3	666.8 ± 60.2	0.01*
OS ratio	1.7 ± 0.1	1.3 ± 0.1	0.05

AH50: alternative hemolytic 50, N: normal activity (⩾80%), PfB: Properdin factor B, C: complement, ORAC: antioxidant defense capacity, MDA: malondialdehyde, OS: oxidative stress, AUC: area under curve.
* Significantly different at $p < 0.05$.

Table 3 Correlations among oxidative stress and complement parameters in SLE patients.

Parameter r(p)	SLE patients (n = 16)	
	AH50	
PfB	0.32	(0.36)
C3	−0.58	(0.02)*
C4	0.06	(0.83)
MDA	−0.52	(0.048)*
ORAC	0.17	(0.56)
	Properin factor B	
C3	−0.79	(0.007)**
C4	0.62	(0.05)
MDA	0.03	(0.94)
ORAC	0.08	(0.82)
	Malondialdehyde	
C3	−0.29	(0.29)
C4	0.29	(0.29)

AH50: alternative hemolytic 50, PfB: Properdin factor B, C: complement, MDA: malondialdehyde, ORAC: antioxidant defense capacity, * = significant at $p < 0.05$ and ** at $p < 0.01$.

4. Discussion

The present study shows that plasma MDA levels and ACP activity were still found to be very high which concords with previous studies [10,32–34]. At present, it is widely accepted that both ACP and MDA are the major mediators of tissue inflammation and SLE progression; On one hand, MDA is associated with disease activity [11]; and on the other hand, ACP represents 80% of complement activity and, is considered as the sole complement pathway involved in SLE tissue damage [13,35].

Importantly, the current study showed that ACP activity significantly correlated with MDA levels which are not in contrast to the evidence that MDA is one of the potent ACP activators. On linear regression 26.8% of ACP activity in SLE may be explained by the increased plasma MDA level as a dependant variable. Of note, it is known that early SLE pathogenesis implicates OS [36] but we wondered if there

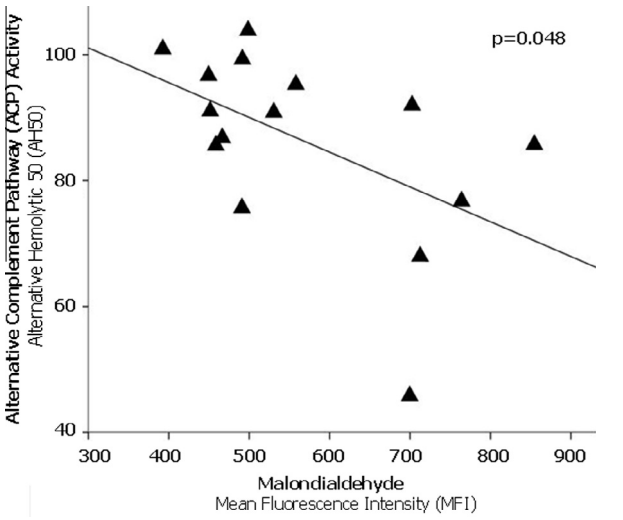

Figure 1 Correlation between the alternative complement pathway activity as expressed by the alternative hemolytic 50 (AH50) with malondialdehyde (MDA) in SLE patients.

was an interesting association with ACP, as described in AMD pathogenesis [12]. Prompted by previous reports about MDA regulation by factor H (FH) in AMD affected eyes, we inferred that OS:ACP interaction might play a key role in SLE pathogenesis. Needless to remind that as the pillar of the immune surveillance, the complement system attacks everything that is not specifically protected. This action is provided by ACP, which is permanently active and constantly probes the environment for the presence of activating surfaces. In OS-related pathologies, oxidized biomolecules and their breakdown products like MDA are generated and, if not properly handled by FH, they activate the ACP [12,25]. Besides, this pathway is characterized by the so-called "amplification loop" that decreases deeply plasma C3 level. Therefore, the data obtained in our study showing that only C3 and MDA correlate with ACP activity is explained by the aforesaid concepts.

Such results would have a particular relevance to current therapeutic approaches and clinical trials based upon antioxidant treatment [37]. Nonetheless, our observations raise questions concerning the long-term use of rituximab, the anti CD20 monoclonal antibody, as a treatment for SLE disease since this therapy is associated with high reactive oxygen species (ROS) generation [38].

As far as we know, this preliminary result is the first report of an eventual association between ACP and MDA in SLE. These findings merit further investigation with larger studies in order to understand exactly the role of OS:ACP interaction on the disease progress, manifestations, activity and outcome in a purpose to develop novel disease preventive biomarkers. Among the limitations of this pilot study is the lack of analysis of clinical manifestations, medications received, disease activity and damage which could be taken into consideration in future studies.

Conflict of interest

None.

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