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ORIGINAL ARTICLE

Atherosclerosis biomarkers in female systemic lupus erythematosus patients with and without cardiovascular diseases

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KEYWORDS

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Abstract *Background:* Cardiovascular diseases (CVD) and atherosclerosis are over presented in patients with systemic lupus erythematosus (SLE).

Aim of the work: The aim of this study is to determine the frequency of some atherosclerosis biomarkers in SLE patients with and without CVD compared with controls.

Patients and methods: 28 female SLE patients with a mean age of 30.1 ± 7.2 years and a history of CVD (SLE cases) were compared with 25 age matched SLE female patients but without a history of CVD (SLE controls) and 25 age matched population based control women (population controls). Intima, media thickness (IMT) was measured by B-mode ultrasound as a potential measure of atherosclerosis. Nontraditional biomarkers of atherosclerosis such as leptin, oxidized LDL (oxLDL) and homocysteine were also investigated.

Results: SLE cases had significantly increased IMT compared with SLE controls and population controls ($p < 0.001$), whereas IMT of SLE controls did not differ from population controls. Compared to SLE controls, SLE cases had raised circulating levels of leptin ($p < 0.001$), homocysteine, dyslipidemia with raised triglycerides ($p < 0.001$), decreased HDL-cholesterol concentration, ($p < 0.001$), lupus anticoagulants ($p = 0.01$), and higher cumulative prednisone dose ($p = 0.4$). Disease duration was comparable between the two SLE groups and the blood pressure and body mass index (BMI) were similar among the 3 groups.

Conclusion: A set of distinct CVD risk factors (biomarkers of atherosclerosis) separate SLE cases from SLE controls and normal population controls. If confirmed in a prospective study, they could be used to identify SLE patients at high risk of CVD in order to optimize treatment.

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1. Introduction

Patients with systemic lupus erythematosus (SLE) have a significantly increased risk of cardiovascular morbidity and mortality, particularly related to premature atherosclerosis (AS). Oxidized LDL (oxLDL) plays an important role in atherogenesis and may contribute to the immune activation and inflammation present in the atherosclerotic lesions, because it has chemotactic, immune-stimulatory, and toxic properties and is taken up by macrophages and other cells in the atherosclerotic plaque, which develop into foam cells [1–6].

Although traditional cardiac risk factors, such as older age, high blood pressure (BP), high cholesterol and triglycerides, smoking, obesity, diabetes mellitus, appear to play a critical role in the determinism of AS, these factors alone cannot adequately explain the increased incidence of cardiovascular disease commonly reported in patients with SLE [1,3]. In Egyptian SLE patients, metabolic syndrome was considered a remarkable risk factor for the development of subclinical atherosclerosis and increased carotid intima-media thickness (IMT) [7–9]. Accordingly, early accelerated AS in SLE is the result of complex cross talk between the usual traditional cardiac risk factors and non-traditional SLE biomarkers of inflammation [1,3,4].

The non-traditional AS biomarkers included both leptin and homocysteine, where leptin acts on the immune system as a proinflammatory cytokine. In animal models, its deficiency is associated with an increased susceptibility to infection and reducing the inflammation [10]. It promotes the proliferation and activation of T lymphocytes and induces production of Th1 cytokines [11–13]. Studies have reported increased leptin levels in SLE patients [14,15]. On the other hand studies stated that homocysteine levels are predictors for the development of coronary artery disease (CAD) and the occurrence of stroke in the general population. In addition, homocysteine levels have been identified as a predictor of atherosclerosis in patients with SLE, in whom high levels may be predictive levels of coronary calcification [16], platelet progression [17] and increased IMT [7,18].

The aim of this study was to determine the frequency of some of the atherosclerosis biomarkers in SLE patients with and without CVD to develop an early screening tool for the identification of high risk SLE patients.

2. Patients and methods

2.1. Study population

We have enrolled 53 SLE patients fulfilling the 1982 revised criteria of the American Rheumatism Association for the classification of SLE [19], with a mean age of 30.5 ± 9.6 years and mean disease duration of 3.6 ± 4.3 years, in addition to 25 age matched healthy populations with a mean age of 30.4 ± 7.1 years. The studied group was classified into two further groups, group (I) which included 28 patients with a history of CVD (SLE cases) and group (II) which included 25 age matched SLE patients without history of CVD (SLE controls). All subjects were informed about the aim of the study and gave their consent. The study was approved by the local ethics committee. The control subjects were unrelated to patients but were ethnically and socioeconomically similar. Physical examinations for them were normal, with blood pressure $<135/85$ mmHg, no urine abnormalities, and no history of autoimmune or rheumatic disease or any other diseases with a known genetic or hereditary predisposition.

All SLE patients were subjected to full history taking, general examination, cardio pulmonary, abdominal, neurological and locomotor systems examination. During routine laboratory investigations (complete blood count, liver and kidney functions by Jaffe kinetic method, and urine analysis), High sensitivity C-reactive protein (hsCRP) was measured using (latex-immunoturbidimetric method: <1 mg/L low risk, 1–3 mg/L medium risk, and >3 mg/L high risk). Immunological assays (ANA and anti-DNA) were done by indirect immunofluorescence and serum C3 and C4 levels by nephelometry (Beckman, USA). Twenty-four hour urine samples were collected to estimate total urinary protein levels by the colorimetric method. Blood and urine samples were always collected on the same day. Detection of the anticardiolipin (ACL) antibodies was done using the enzyme linked immunosorbent assay (ELISA), while detection of lupus anticoagulant was done using the dilute Russel viper venom time (dRVVT) clotting assay.

Systemic hypertension was recorded when systolic $\geqslant 140$ mmHg and/or diastolic blood pressure $\geqslant 90$ mmHg, measured in multiple occasions or when antihypertensive medication was taken. Dyslipidemia was defined as any of the following or in combination: raised low density lipoprotein (LDL) cholesterol >130 mg/dl, total cholesterol >200 mg/dl, triglycerides >150 mg/dl, or low level of high density lipoprotein (HDL) cholesterol <40 mg/dl. Diabetes mellitus was defined either by fasting blood sugar >120 mg/dl or taking insulin or hypoglycemic drugs. The global disease activity was assessed by SLE disease activity index (SLEDAI) [20].

All patients were taking steroids (dose range 15–50 mg/day), 45 patients on hydroxychloroquine (dose range 200–400 mg/day), 25 patients on azathioprine (dose range 100–150 mg/day) and 18 patients were receiving monthly cyclophosphamide pulse therapy depending on extent of renal lesion (dose range 700–1000 mg).

2.2. Measurement of serum biomarkers

Clotted samples were stored at -20 °C until the time of analysis.

- Oxidized LDL was assayed using kit purchased from immunodiagnostic AG (catalog No. K7810). That was a sandwich ELIZA for direct measurement of oxLDL in human serum. Standards, controls and samples containing human oxLDL were added to wells of microplate that were coated with high affinity antibodies. A dose response curve of absorbance unit (optical density at 450 nm) versus concentration was generated; using the values obtained from standard. oxLDL present in the patient samples was determined directly from this curve. Patients taking statins in the past 3 months were excluded.
- Leptin was analyzed by leptin kit, (DRG instruments GmbH, division of DRG International, Inc, *Vers*. 9.0, *Ref*: EIA-2395, Germany) through ELIZA.