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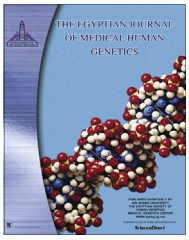

ORIGINAL ARTICLE

Circulating cell free DNA as a predictor of systemic lupus erythematosus severity and monitoring of therapy

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Abstract *Background:* Systemic lupus erythematosus (SLE) is the most heterogeneous chronic autoimmune disease; it is characterized by the presence of auto reactive B and T cells, responsible for the aberrant production of a broad and heterogeneous group of autoantibodies. Recent studies using various detection methods have demonstrated the elevations of circulating DNA in SLE patients.

Aim of the study: The current study aimed to measure cell-free DNA (cf-DNA) in SLE patients as a potential tool to predict disease activity and treatment follow up.

Subjects and methods: 52 of SLE patients with age ranging from 10 to 48 years were randomly selected and 25 healthy subjects with age and gender matched with the patients were included as a control group. Thorough clinical examination stressing on the central nervous system, vascular, renal, rash, musculoskeletal, mucocutaneous manifestations, and fever was done for patients. The following investigations were done: Complete blood count (CBC), kidney function tests, C-reactive protein (CRP), routine autoantibodies for autoimmune diseases, complements (C3 & C4), anti-nucleosome antibodies and cf-DNA by real time PCR (RT-PCR).

Results: The levels of anti-double stranded DNA (anti-dsDNA), anti-nucleosome Ab, and cf-DNA were significantly increased in SLE patients compared to controls. The cf-DNA level was correlated to markers of disease severity namely CRP and anti-nucleosome. A significant reduction in levels of cf-DNA, anti-nucleosome Ab and anti-dsDNA was noticed after therapy.

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Conclusion: Our findings support that the measurement of cf-DNA appears to be a useful marker in addition to laboratory tests used in SLE diagnosis. High correlation with markers of disease severity suggesting its role in disease pathogenesis and decreasing its level after therapy makes it to be a marker of treatment follow-up.

1. Introduction

Circulating cell-free DNA (cf-DNA) has been found in the plasma of human subjects. It has been extensively studied over the past few decades. Supported by theory and observation, two major sources of cf-DNA have been postulated: first, fragmented DNA released as a consequence of cell death (apoptosis/necrosis of blood and tissue cells) and, second, active metabolic secretion of DNA from cells. Considerable research efforts had been made on the use of cf-DNA as a biomarker in cancer diagnosis [1].

In various pathologic conditions, qualitative and quantitative changes in circulating DNA have also been shown, such as mutations, deletions, methylations and microsatellite aberrations which are distinct from those in benign conditions, and thus may be useful in the diagnosis of cancer [2]. Only small amounts of serum or plasma DNA have been observed in healthy individuals, whereas high concentrations had been described in patients with various malignancies and in those with several benign diseases, such as infections, sepsis, trauma, stroke, and autoimmune diseases [3,4]. Because most of these disorders are associated with increased rates of cell death events, from apoptosis or necrosis, these mechanisms are considered to be the main sources for circulating DNA. Active release of DNA by lymphocytes is thought to be of minor relevance [4,6].

For many years, free DNA research has been focused on examining the level of free DNA in autoimmune diseases like rheumatoid arthritis [7], systemic sclerosis [8] and primary Sjogren's syndrome [9]. In case of rheumatoid arthritis, Leon et al. [7] had discovered higher concentrations of free DNA in both plasma and synovial fluid than in healthy subjects while the increasing intensity was correlated with the symptom severity and the level of the tissue damage. Unlike them, Mosca et al. [8] did not establish the significant difference in cf-DNA concentration in patients with systemic sclerosis and in healthy subjects, but based on the cf-DNA level they could make a difference between patients with active disease and those with the inactive one.

Systemic lupus erythematosus (SLE) is an autoimmune disease that has the potential of affecting multiple organ systems, including the skin, muscles, bones, lungs, kidneys, as well as the cardiovascular and central nervous systems [10,11].

SLE can cause various tissue inflammation and damage in a chronic manner and cell death [12]. Cell death has been regarded as an important event in the pathogenesis of SLE, as it leads to the release of antigens, such as nucleic acids, for immune complex formation, and that DNA-antibody complexes in the circulation are one of the hallmarks of SLE. DNA-antibody complexes may trigger a cascade of immune responses against the bodily tissues of the SLE patients [13,14].

SLE was one of the pathological conditions reported to be associated with the presence of circulating DNA nearly 59 years ago [15]. Since then, studies using various detection methods have demonstrated the elevations of circulating DNA in SLE patients [16,17]. Investigating whether and to what degree fluctuations in cf-DNA levels in patients with SLE might correspond to disease severity was the goal of many investigations [8,17]. The most recent data seem to exclude measuring cf-DNA as an inexpensive, simple and quick tool to assess disease activity in patients with SLE [9,17].

The application of molecular biologic techniques has allowed the molecular characterization of cf-DNA in certain pathologic and physiologic conditions. Various methods have been established for the measurement of circulating DNA. Quantification of DNA in plasma and serum by real-time PCR is widely accepted as standard and detects all kinds of free and protein bound circulating DNA [5]. However, there have been very few studies reporting the detailed biological characterization of circulating DNA in SLE [18]. The extremely variable clinical manifestations and the absence of effective tests to monitor disease activity present a challenge for clinical management.

1.1. Aim of the study

The current study aimed to measure the circulating cell free DNA (cf-DNA) as a potential tool to predict disease severity and treatment follow up in patients with SLE.

2. Patient and methods

Fifty-two of SLE patients attending the Menoufiya and Al Azhar University Hospitals between February 2013 and September 2014 were included in the study. Samples were taken from patients who fulfilled at least four of the American College of Rheumatology criteria for the diagnosis of SLE [19]. They were 40 females and 12 males with age ranging from 10–48 years, mean ± SD (28.42 ± 11.43). Twenty-five healthy subjects (18 female and 7 males) age and gender matched with the patients were included in the study as a control group.

All patients were subjected to: full history and thorough clinical examination stressing on the central nervous system, vascular, renal, rash, musculoskeletal, mucocutaneous manifestations, and fever. Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) was calculated for all patients, a score of 8 or more was defined as active disease at the beginning of the study [20]. Fourteen patients had SLEDAI score of 6 or less and hence were considered inactive SLE; the remaining 38 were categorized to have active SLE disease.