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Case Report

DNA damage in a patient with Systemic Lupus Erythematosus and Nephropathy- A Case Report

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Abstract

Objectives: Systemic Lupus Erythematosus (SLE) is a complex, multisystem autoimmune inflammatory disease. The inflammatory response in SLE exacerbates oxidative stress which tends to promote lipid peroxidation, protein oxidation and damage to DNA. Documentation of manifestation of DNA damage in SLE patients is scarce and related studies have not come to attention from this part of the world, therefore the present study showcases a case report on SLE.
Methods: Assessment of DNA damage in peripheral blood leukocytes and oxidative stress biomarkers in blood sera of a 12y-old male presenting with SLE and nephropathy (on dialysis therapy) was made.
Results: Basal DNA damage in PBL scored as percent DNA in tail was higher (51.48%) compared to levels (40.38%) in an age-and sex-matched healthy control. Quantitative measures of DNA damage revealed DF of 96 *vs.* 94 and DI of 317 *vs.* 208 in the case and the control, respectively. Serum lipid peroxidation as estimated from MDA level was 3x higher (1.759µmol/l) compared to its level in a healthy control (0.571µmol/l). OSI was slightly higher in the patient (0.134 arbitrary units) compared to that in the control (0.132 arbitrary units). The atherogenic indices were higher in the present case compared to ratios in the control.
Conclusion: The results from the case report on increased oxidative stress, dyslipidemia, genetic damage and atherogenic indices support observations from earlier studies and imply onset of other complications in patients with SLE. The assessment of various blood-based biomarkers (as carried out in the present case) in SLE patients may assist in disease diagnosis, prognosis and optimal disease management.
Keywords: Genetic damage, lupus nephritis, oxidative stress.

1. Introduction

Systemic Lupus Erythematosus (SLE) is a multisystem autoimmune inflammatory disease of the connective tissue with a worldwide prevalence of 2–140/100,000 [1] and of 14-60 per 100,000 in India [2]. The disease has a multifactorial pathogenesis involving environmental and genetic factors [3]. Environmental triggers include sunlight-exposure, drugs and infections [4]. The disease has a 10% hereditary component and genetic association studies have revealed more than 25 candidate genes which contribute to the mechanisms that predispose individuals to lupus [2]. Relapses, flares and remissions with variable manifestations which include malar rash, cutaneous photosensitivity, nephropathy, serositis and polyarthiritis are characteristics of the disease [5]. Prescribed medications comprise immunosuppressive drugs and nonsteroidal anti-inflammatory drugs (NSAIDs); however introduction of mycophenolate salts and Rituximab has refined the drug treatment therapy [6]. Disease-prognosis includes lupus reactivations, infections, vascular access thrombosis and cardiovascular complications [6] while 60% of SLE cases develop nephropathy with 10-28% requiring dialysis [6,7]. In fact, SLE patients with renal failure (lupus nephritis) minimally require dialysis therapy for three months before receiving a kidney transplant [8].

The inflammatory response in SLE exacerbates oxidative stress [9] which tends to promote cellular macromolecular damage, lipid peroxidation and protein oxidation [10,11] and damage to DNA [12,13]. Health can further be compromised as any unrepaired DNA damage can initiate neoplasia [14].

As documentation of manifestation of DNA damage in SLE patients is scarce and related studies have not come to attention from this part of the

world, the present study showcases a case report on SLE. The study has importance given the low SLE prevalence in India and therefore insights from the study results can provide a base-line for a larger study-sample to assist in disease-diagnosis and prognosis. The present case-study hence investigated level of DNA damage and oxidative stress in peripheral blood leukocytes of a 12y-old male presenting with SLE and nephropathy requiring dialysis therapy for management and sustaining life. Written informed consent from the parents and ethical approval as a part of a research project from the Institutional Ethics Committee was obtained before carrying out the study.

2. Case Report

A 12 year old male (born to primigravida) diagnosed with SLE was admitted to a local hospital with kidney-care facilities after he had fainted in a sports` event while playing in the ground in the afternoon hours. He presented with symptoms of nausea, blood vomiting, breathlessness, loose motions, redness of sclera, rashes on cheeks and hematuria of past three days. Biochemical investigations revealed increased levels of serum creatinine (3.8 mg/dl) and urea (113 mg/dl). The Cock-Croft Gault equation [15] was used to calculate the glomerular filtration rate (GFR) which was significantly far lower (22.46 ml/min/1.73m^2) than the normal GFR value of 120 ml/min/1.73m^2, and hence immediately required hemodialysis therapy.

Demographic information revealed that the patient studied in grade seven and the parents belonged to upper middle socio-economic status calculated as per Kumar *et al*[16]. Dietary preference included non-vegetarian food and had a BMI of 21.42 kg/m^2 which was within the normal range for his age [17]. Serum creatinine level was almost 2.5x higher than the upper normal range (0.8-1.4 mg/dl) while urea levels were 5.65x (113 mg/dl) the upper normal range value (8-20 mg/dl). Severe anemia was also present as Hb level was only 8mg/dl.

Basal DNA damage was assessed in the peripheral blood leukocytes while oxidative stress and dyslipidemia were assessed in peripheral blood serum sample. The micro-electrophoretic single cell gel electrophoresis (SCGE) assay [18] was used for assessment of DNA damage which discerns single-strand and double-strand DNA breaks, alkali-labile sites, cross-links and apurinic sites at cell level on alkaline electrophoresis [19]. Briefly, 30 µl of blood mixed with low melting point agarose was sandwiched between agarose layers, lysed (lysis buffer- 2.5M NaCl, 100mM EDTA, 10mM Tris, 1% Triton X-100, 10% DMSO; pH 10 adjusted with NaOH) and electrophoresed. The slides were stained with silver nitrate [20], coded and scored blind (50 cells/slide; 2 slides/sample) for comets using the freely available cell image analysis software (CellProfiler r11710) for per cent DNA in tail. The per cent DNA in tail is a measure of amount of DNA in comet tail. Depending on the length of the tail, the nucleoids were categorized into damage classes [21] and Damage frequency (DF) and Damage Index (DI) were also calculated [22]. DF is number of nucleoids with tails and DI indicates summation of damage classes of 100 cells analyzed per individual.

Standard spectrophotometry was used for assessment of blood sera samples for oxidative stress parameters of Malondialdehyde (MDA) [23] which is an end-product of lipid peroxidation, total antioxidant capacity (TAC) [24] and total oxidant status (TOS) [25]. MDA levels are an indirect measure of lipid peroxidation while TAC corresponds to synergestic effect of all antioxidants viz. alpha and beta carotene, lycopene, Vitamins C and E and TOS corresponds to all oxidants present in the body [26].

Lipid profiling was carried out on a semi-automated blood analyser (ERBA Mannheim Diagnostic, Germany) using standard kits (ERBA, HP). Levels of total cholesterol (TC), high density lipoprotein cholesterol (HDL-C) and triglycerides (TG) were determined. Low density (LDL) and very low density lipoprotein cholesterol (VLDL-C) levels were calculated using Friedewald equation [27]; the atherogenic indices (TC/HDL-C, LDL-C/HDL-C, TG/HDL-C) were also evaluated.

3. Results

Basal DNA damage in PBL scored as percent DNA in tail was higher (51.48%) compared to levels (40.38%) in an age- and sex-matched healthy control. Quantitative measures of DNA damage revealed DF of 96 *vs.* 94 and DI of 317 *vs.* 208 in the case and the control, respectively. Comparison of cell damage categories on Chi-square analysis highlighted significantly higher category of classes I ($p=0.000$), III ($p=0.033$) and IV ($p=0.014$) comets in the case compared to those in the control.

Serum lipid peroxidation as estimated from MDA level was 3x higher (1.759µmol/l) compared to its level in a healthy control (0.571µmol/l). Surprisingly, both sera TAC (15.54 mmolEq/l) and TOS (2.059 mmolH_2O_2Eq/l) levels were increased in the control compared to respective levels in the case (4.648 mmolEq/l and 0.624 mmol H_2O_2Eq/l). However, OSI was slightly higher in the patient (0.134 arbitrary units) compared to that in the control (0.132 arbitrary units). Serum TC (172 mg/dl;

normal range 140-250 mg/dl) and TG levels (110.80 mg/dl; normal range 25-160 mg/dl) were in the normal range while HDL-C level was decreased (24.70 mg/dl; normal range 30-65 mg/dl) in the patient. LDL-C and VLDL-C levels were 125.14 *vs.* 145.81 and 22.16 *vs.* 14.69 in the case vs. control, respectively. The atherogenic indices were higher {6.96 *vs.* 3.48 (TC/HDL-C), 5.07 *vs.* 2.26 (LDL-C/HDL-C), 4.48 *vs.* 1.14 (TG/HDL)} in the present case compared to ratios in the control.

4. Discussion

SLE is an autoimmune inflammatory disease affecting multiple organs commonly the skin, joints, haemopoietic system, kidneys, lungs and central nervous system [28]. The present report to the best of our knowledge the only of its kind, documents DNA damage in a 12y male case with SLE nephropathy. Although aetiology of SLE is multifactorial, yet the exact pathological mechanisms of SLE remain elusive [3]. As similar studies on genetic damage and oxidative stress in SLE patients in literature are lacking, the discussion has been extended to include cytotoxicity/apoptosis and associated co-morbidities in SLE disease. Bijl *et al* [29] had demonstrated abnormal phagocytosis in SLE patients showing reduced uptake of apoptotic cells by monocyte-derived macrophages. The redox state gets altered causing abnormal activation of apoptosis [30] which probably manifests as clinical manifestations of redness of sclera, rashes on cheeks etc. as also observed in the present case.

Nephritis has tendancy to induce oxidative stress via inflammation [31] which also is a characteristic feature of SLE [9]. Oxidative stress has also been observed also in the present case. In fact elevated MDA levels and reduced TAS levels also point towards a state of oxidative stress. Shah *et al* [9] have recently reported inflammatory response in SLE which aggravates oxidative stress with potential to provoke damage to lipids, proteins and DNA [12, 13]. The observations of the present study on significantly increased genomic damage also find reflections in the results documented by Montalvao *et al* [13] who reported 1.3x significantly increased (p=0.000) DNA damage index in 25 SLE patients compared to the value in equal number of healthy controls.

The results from the case report on increased oxidative stress, dyslipidemia, genetic damage and atherogenic indices support observations from earlier studies and imply the onset of other complications in patients with SLE. The assessment of various blood-based biomarkers (as carried out in the present case) in SLE patients may assist in disease diagnosis, prognosis and optimal disease management.

The observed genetic damage may be attributable from persistent oxidative stress and protein oxidation in SLE patients as OSI, which is ratio of TOS to TAC and 3x increases in lipid peroxidation was observed. The observed genetic damage may also be contributed by increased serum creatinine levels (which were 2.5x higher). Stoyanova *et al* [32] had also observed a significant positive correlation between serum creatinine levels and genomic damage being substantiated by observations of the present study.

Furthermore, SLE is associated with an increased risk for development of atherosclerosis and cardiovascular disease [3,33]. In the present case, higher atherogenic indices of TC/HDL-C, LDL-C/HDL-C, TG/HDL-C compared to the control which also imply risk for cardiovascular complications.

The consequences of oxidative modification in SLE play a crucial role in immunomodulation and trigger autoimmunity [9] as also which probably occurred in the present case. In fact measurements of oxidative modifications in biological samples from SLE patients may assist in the elucidation of the pathophysiological mechanisms of the oxidative stress-related damage, disease prognosis and adequate treatment strategy in the early stages.

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