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DNA damage and oxidative stress in patients with mild preeclampsia and offspring

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ABSTRACT

Objective: Oxidative stress has been shown to play an important role in the pathogenesis of pre-eclampsia, and DNA damage frequently occurs in cells exposed to such stress. The aim of the present study was to investigate DNA damage and oxidative stress in mildly pre-eclamptic women and their offspring.
Study design: We studied 25 mildly pre-eclamptic mothers, 20 healthy controls, and their infants. Mononuclear leukocyte DNA damage, total antioxidant status (TAS), and total oxidant status (TOS) were determined and the oxidative stress index (OSI) was calculated.
Results: DNA damage, and TOS and OSI levels were significantly increased, and TAS levels significantly decreased, in maternal and cord blood samples of the mildly pre-eclamptic group. A positive correlation between the extent of DNA damage and diastolic blood pressure was evident in pre-eclamptic mothers and there was a negative correlation between the extent of DNA damage and TOS.
Conclusion: Both oxidative stress and DNA damage are elevated in mildly pre-eclamptic patients and their offspring. Increased oxidative stress may be important in inducing DNA damage in pre-eclamptic patients.

1. Introduction

Pre-eclampsia is a complex multisystemic disorder and is associated with the highest maternal and fetal morbidity and mortality of all pregnancy complications [1]. It is recognized that injury to the vascular endothelium is a basic pathological event in pre-eclampsia [2] and such endothelial damage is mediated by oxidative stress imposed by increased generation of oxygen free radicals or a fall in antioxidant levels [3]. Patients with pre-eclampsia have been shown to be under increased oxidative stress [4] and oxygen free radicals created by such stress induce several types of DNA damage [5]. Many pathological conditions including various cancers, cardiovascular and neurodegenerative diseases, inflammation/infection, and aging are associated with DNA damage [6,7].

Several methods have been employed to monitor genetic damage to mononuclear leukocytes as an indicator of general genetic damage. These include the micronucleus (MN) test, analysis of chromosomal aberrations, the sister-chromatid exchange (SCE) test, gene mutation tests, and the comet assay [8]. Of these tests, the comet assay (single-cell gel electrophoresis) is a well-established genotoxicity test that is simple, rapid and sensitive; the test has been used to assess the extent of endogenous DNA damage [8,9]. The assay has not been used, however, to gather information on any possible peripheral DNA damage in women with mild pre-eclampsia. In the present study, therefore, we explored DNA damage and oxidative marker levels in mildly pre-eclamptic women and their offspring to determine if DNA damage and oxidant status were associated in women with this syndrome.

2. Materials and methods

2.1. Subjects

This cross-sectional study was conducted at the Department of Obstetrics and Gynecology at Harran University School of Medicine and Sanliurfa Women's Health and Maternity Hospital between January 2012 and August 2012. Twenty-five hypertensive pregnant women (mildly pre-eclamptic) and 20 normotensive pregnant women were included. Mild pre-eclampsia was considered

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present when a diastolic blood pressure of 90 mm Hg or greater was measured on two occasions at least 6 h apart and when proteinuria was evident; proteinuria was defined as two or more positive dipstick test results for protein, or >300 mg total protein in a 24-h urine collection [10].

All subjects were nonsmokers, were not taking any medication, had no other disease, and had singleton pregnancies. All pregnancies were uneventful and healthy infants were born vaginally at term. Members of both study groups were recruited within 2 h of admission from patients with spontaneous labor onset. All subjects were in the early phases of labor. Blood samples were taken when cervical dilatation was less than 3 cm. Of all women, those who experienced any problem in any phase of labor, and whose babies had Apgar scores below 8, were excluded from the study. Subjects were also excluded if they had severe pre-eclampsia, defined as a systolic blood pressure of 170 mm Hg or higher, a diastolic blood pressure of 110 mm Hg or higher, or proteinuria of 5 g or more per 24 h. Other exclusion criteria were pre-existing hypertension treated with antihypertensive drugs, diabetes mellitus, gestational diabetes, renal disease, heart disease, a previous cesarean section, HELLP syndrome, oliguria of less than 500 ml per 24 h, pulmonary edema or cyanosis, fetal anomalies, suspected intrauterine growth restriction (IUGR), and abnormalities detected during fetal heart-rate monitoring.

All participants were informed about the study protocol and written consent was obtained from each woman. The study protocol conformed to the principles of the Helsinki Declaration and was approved by the Medical Ethics Committee of Harran University. Peripheral venous blood samples were taken at the early latent phase when the amniotic membrane was intact and the fetus was in cephalic presentation. Umbilical cord blood samples were taken just after delivery.

2.2. Blood collection and storage conditions

Blood samples (~5 ml) were collected from the antecubital vein of mothers and from the umbilical cord just after delivery. Samples were immediately transferred into heparinized tubes, stored at 2–4 °C in the dark to prevent further DNA damage, and processed within 2 h. Mononuclear leukocytes were isolated by centrifugation on Histopaque 1077 (Sigma). One milliliter amounts of heparinized blood were carefully layered over 1 ml amounts of Histopaque and centrifuged for 35 min at 500 × *g* at 25 °C. Each interface band containing mononuclear leukocytes was washed with phosphate-buffered saline (PBS) and collected by 15-min centrifugation at 400 × *g*. The resulting pellets were resuspended in PBS and cells were counted using an automatic cell counter (Abbott 3700, USA). Membrane integrity was assessed using the Trypan-Blue exclusion assay. The remaining blood was centrifuged at 1500 × *g* for 10 min to obtain plasma which was stored at −80 °C prior to analysis of total oxidant status (TOS) and total antioxidant status (TAS).

2.3. Determination of DNA damage using the alkaline comet assay

The comet assay, also known as the single-cell gel electrophoresis (SCGE) assay, was performed as described by Singh et al. [8,11] with the following modifications: 10 μl amounts of fresh mononuclear leukocyte cell suspensions (roughly 20,000 cells) were mixed with 80 μl of 0.7% low melting-point agarose in PBS at 37 °C. Next, 80 μl of each mixture was layered onto a slide precoated with a thin layer of 1% normal melting-point agarose (NMA) and immediately covered with a cover slip. Slides were held for 5 min at 4 °C to allow the agarose to solidify. After removal of cover slips the slides were immersed in freshly prepared cold (4 °C) lysing solution (2.5 M NaCl, 100 mM Na_2EDTA, 10 mM Tris–HCl, 1% Triton X-100, and 10% DMSO [added just before use]; pH 10–10.5) for at least 1 h. Slides were next immersed in freshly prepared alkaline electrophoresis buffer (0.3 M NaOH and 1 mM Na_2EDTA; pH > 13) at 4 °C to allow DNA to unwind (40 min) and then electrophoresed (25 V/300 mA, 25 min). All manipulations were performed under minimal illumination. After electrophoresis, the slides were neutralized (0.4 M Tris–HCl; pH 7.5) for 5 min.

Dried microscope slides were stained with ethidium bromide (2 μg/ml in distilled water; 70 μl/slide), covered with cover slips, and viewed by fluorescence microscopy (Olympus BX51, Japan) at 200x magnification. The microscope was capable of detecting epifluorescence and was equipped with a rhodamine filter (excitation wavelength 546 nm; barrier 580 nm). The extent of extranuclear fluorescence was scored (by eye) in 50 random cells of each sample using a scale of 0–4 as previously described by Kobayashi et al. [9,12]. Scoring was as follows: 0, no tail; 1, comet tail < half the width of the nucleus; 2, comet tail equal to the width of the nucleus; 3, comet tail longer than the width of the nucleus but not twice as long; 4, comet tail > twice the width of the nucleus. This type of scoring has been shown to be as accurate that afforded by computerized image analysis [9]. All slides were coded and were scored in a blinded manner. A visual score for each class of subjects was calculated by multiplying the percentages of cells in the various comet classes by the score for that class. The total visual comet score reflecting the extent of DNA damage was the sum of scores for all five comet classes. Thus, a total visual score could range from 0 (all undamaged) to 400 (all maximally damaged) in arbitrary units (AU).

2.4. Measurement of total oxidant status

Plasma TOS was measured using a novel automated method developed by Erel [13]. Oxidants present in a sample oxidize the ferrous ion of an o-dianisidine complex to ferric ion. Oxidation is enhanced by glycerol, which is abundant in the reaction medium, and the ferric ion forms a colored complex with xylenol orange under acidic conditions. Color intensity (which can be measured spectrophotometrically) is associated with the total level of oxidants present. Hydrogen peroxide is used to calibrate the assay and results are expressed in terms of micromoles of hydrogen peroxide equivalent per liter (μmol H_2O_2 equiv./l).

2.5. Measurement of total antioxidant status

Plasma TAS was measured using another novel automated method developed by Erel [14]. This involves production of the hydroxyl radical, which is a potent biological reactant. A ferrous ion solution (Reagent 1) is mixed with hydrogen peroxide (Reagent 2). Radicals produced by the hydroxyl radical, including the brown dianisidinyl radical cation, are also potent in biological terms. Thus, it is possible to measure the antioxidative capacity of a sample in terms of inhibition of free radical reactions initiated by production of the hydroxyl radical. Variation in assay data is very low (less than 3%) and results are expressed as mmol Trolox equiv./l.

2.6. Measurement of oxidative stress index

The OSI was the TOS-to-TAS ratio, but TAS values were changed to mmol/l. Each OSI was calculated as follows: OSI (arbitrary units) = TOS(μmol H_2O_2/l)/TAS(mmol Trolox/l) [15].

2.7. Statistical analysis

The Statistical Package for Social Sciences (SPSS 15.0; SPSS Inc., Chicago, IL) was used in all statistical analyses. Application of the Kolmogorov–Smirnov test revealed that data distributions were