Increased oxidative stress in patients with hydatidiform mole

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Summary

Objective: The aim of this study was to determine the oxidative status and antioxidative status of plasma of patients with complete hydatidiform mole (CHM) and to compare these values with normal pregnancy.

Method: Thirty-eight patients with CHM and 31 healthy pregnant women were enrolled in the study. To determine the antioxidative status of plasma, total antioxidant potential (TAOP) was calculated, and to determine the oxidative status of plasma total peroxide levels were measured. The ratio of TAOP to total peroxide was accepted as an indicator of oxidative stress.

Results: TAOP of plasma was significantly lower in patients with hydatidiform mole than in healthy pregnant women [mean (SD) values were 511.9 (105.8) and 571.7 (109.4) µmol Trolox equiv./L respectively (p <0.05)]. In contrast, mean (SD) total peroxide level of plasma was significantly higher in the patients [21.8 (6.4) µmol H2O2/L] than in healthy pregnant women [15.6 (6.4) µmol H2O2/L (p <0.001)]. The mean oxidative stress index level was significantly higher in patients with CHM than in healthy pregnant women [4.43 (1.70) versus 2.92 (1.50) respectively (p <0.001)].

Conclusion: Patients with CHM are exposed to oxidative stress, which may have a role in the pathogenesis of the disease. Supplementation with antioxidative vitamins such as C and E could be considered in treatment.

Key words: antioxidant; total antioxidant; total antioxidant potential; total peroxide; hydatidiform mole; pregnancy

Introduction

Reactive oxygen species (ROS) are produced in metabolic and physiological processes, and harmful oxidative reactions may occur in organisms which remove ROS via enzymatic and non-enzymatic antioxidative mechanisms. Under some conditions increases in oxidants and decreases in antioxidants cannot be prevented, and the oxidative/antioxidative balance shifts towards the oxidative status. Oxidative stress, which has been implicated in over 100 disorders, develops in consequence [1].

Blood contains many antioxidant molecules that prevent and/or inhibit harmful free radical reactions [2]. Plasma concentrations of antioxidants can be measured separately in the laboratory, but these measurements are time-consuming, labour-intensive and costly. Since antioxidative effects of antioxidant components of plasma are additive, the measurement of total antioxidant potential (TAOP) reflects the antioxidative status of plasma. We evaluated the total antioxidative status of plasma with TAOP [3, 4].

Hydrogen peroxide and other derivatives of peroxides, produced physiologically in organisms and occurring in higher concentrations in some pathological conditions, diffuse into plasma. Here, antioxidant components of plasma overwhelm them and they are consumed [5]. We evaluated the total oxidative status of plasma by measuring total peroxide level [6].

It is known that oxidative stress increases during normal pregnancy. In healthy pregnancy it has been reported that plasma lipid hydroperoxide levels are increased and total antioxidant capacity decreased [7], while erythrocyte glutathione peroxidase activity and its cofactor selenium are diminished [8]. However, the nature of this mechanism is not yet known.

Pre-eclamptic patients are exposed to increased oxidative stress [9, 10], as are patients with complicated pregnancies such as those involving hypertension and diabetes mellitus [11, 12]. Placental hypersecretion of lipid peroxides or decreased placental antioxidant enzyme production
may lead to endothelial dysfunction. Insufficient antioxidant capacity may lead to excess oxidative stress; oxidative injury may subsequently occur in maternal and placental compartments [13]. Thus, in pre-eclampsia, placental abnormality and associated metabolic changes cause increased oxidative stress [14]. Similar metabolic changes are present in molar pregnancy.

We postulated that increased oxidative stress may be present in patients with CHM, and in the present state of knowledge there has been no report involving the oxidative/antioxidative status of plasma in the pseudopregnant state of CHM. This study is an attempt to add to that body of knowledge.

Materials and methods

Subjects

This study involved 69 women who attended Harran University Hospital during the period between July 1998 and September 2002. Of these, 31 were healthy pregnant women (controls) in the first trimester of pregnancy with a single viable foetus (mean gestational age 13.2 weeks as estimated by ultrasonography). The remaining 38 patients had CHM (mean gestational age 12.9 weeks as estimated by last menstrual period). Obese women, underweight women and smokers were excluded from the study. Diagnosis of complete hydatidiform mole was based on histopathological examination of molar tissue, showing characteristically abnormal proliferation of trophoblastic tissue, lack of an identifiable foetus, chorionic villi with generalised hydatidiform swelling, and diffuse trophoblastic hyperplasia resulting from abnormal fertilisation. Informed written consent was obtained from all subjects.

Samples

Blood samples were withdrawn into heparinised tubes after overnight fasting. Plasma was separated from cells by centrifugation at 1500 g for 10 min. Plasma samples were assayed immediately or stored at –80 °C.

Measurement of plasma total antioxidant potential

TAOP of plasma was determined using the ferric reducing/antioxidant power (FRAP) assay, developed by Benzie and Strain [3, 4]. Briefly, 1.5 ml of working prewarmed 37 °C FRAP reagent (10 vols 300 mM acetate buffer, pH 3.6 + 1 vol. 10 mM 2,4,6-tripyridyl-S-triazine in 40 mM HCl + 1 vol. 20 mMol FeCl3) was vortex mixed with 50 µL test sample and standards. The test was performed at 37 °C. Absorbance at 593 nm was read against a reagent blank at a predetermined time (0–4 min) after sample-reagent mixing. An arbitrary unit, the “Trolox equivalent” concentration, was used as the measurement of TAOP [15]. Thus the results were expressed as µmol Trolox equiv./L [3, 4, 7].

Measurement of plasma total peroxide concentration

Total peroxide concentrations were determined using the “FOX2” method [6] with minor modifications. The FOX2 test system is based on oxidation of ferrous ion to ferric ion by various types of peroxides contained within the plasma samples, to produce a coloured ferric–xylenol orange complex whose absorbance can be measured. The FOX2 reagent was prepared by dissolving ammonium ferrous sulphate (9.8 mg) in 250 mM H2SO4 (10 ml) to give a final concentration of 250 µM ferrous ion in acid. This solution was then added to 90 ml HPLC-grade methanol containing 79.2 mg butylated hydroxytoluene (BHT). Finally, 7.6 mg xylenol orange was added with stirring to make the final working reagent (250 µM ammonium ferrous sulphate, 100 µM xylenol orange, 25 mM H2SO4, and 4 mM BHT in 90% vol/vol methanol in a final volume of 100 ml). The blank working reagent contained only ferrous sulphate.

Aliquots (200 µL) of plasma were mixed with 1800 µL FOX2 reagent. After incubation at room temperature for 30 min, the vials were centrifuged at 12000 g for 10 min. Absorbance of the supernatant was then determined at 560 nm. Total peroxide content of plasma samples was determined as a function of the absorbance difference between test and blank tubes using a solution of H2O2 as standard. The coefficient of variation for individual plasma samples was less than 5%.

Oxidative stress index

The ratio of total peroxide to total antioxidant potential was the oxidative stress index, an indicator of the degree of oxidative stress.

Statistical analysis

Student’s t test was performed using SPSS package. P ≤0.05 was considered statistically significant.

Results

Demographic and clinical data of the subjects are shown in table 1. There were no differences in mean age, gestational age, gravidity, parity, abortion and body mass index (BMI) between patients with CHM and controls.

As seen in table 2, plasma TAOP levels of patients with CHM were found to be significantly lower than those of healthy pregnant women: 511.9 (105.8) vs 571.7 (109.4) µmol Trolox equiv./L (p <0.05). Plasma total peroxide levels were significantly higher in hydatidiform mole patients than in controls: 21.8 (6.4) vs 15.6 (6.4) µmol H2O2 (p <0.001). OSI was significantly higher in complete hydatidiform patients than in controls: 4.43 (1.70) vs 2.92 (1.50) (p <0.001).
In the present study, we found that the oxidative/antioxidative balance shifted towards oxidative status, namely increased oxidative stress was present in patients with CHM compared with healthy pregnant control subjects. Further studies are needed to explain the exact mechanisms of oxidative stress in patients with CHM. We postulated that the hypothetical mechanisms in pre-eclampsia may also be responsible for CHM. In both diseases the placenta is a key source of factors which lead to similar metabolic changes [10].

In pre-eclampsia leucocytes, such as neutrophils and monocytes, are activated. Inflammatory cytokines, such as interleukin-6 (IL-6) and tumour necrosis factor (TNF(\(\alpha\))), and the vascular cell adhesion molecule (VCAM-1), are elevated in the maternal circulation. Activated neutrophils attach to endothelial cells where they generate ROS, resulting in oxidative stress within the cell and internal milieu [14]. In CHM disease, similar inflammatory mechanisms to those mentioned above are present [16, 17]. Increased ROS and decreased antioxidative defence systems may also lead to oxidative injury in patients with CHM.

As is seen in table 2, we found that TAOP was decreased and the total peroxide level increased in these patients and, as expected, they are exposed to increased oxidative stress. Increased oxidative stress may play a role in the pathogenesis of the disease or may be secondary to the disease. Supplementation with antioxidant vitamins C and E may prove useful in the treatment of CHM.

**Discussion**

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