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May supplementation of coenzyme Q10 help prevent development of hydatidiform mole?

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Summary

Objective: The pathological mechanisms of gestational trophoblastic disease have not yet been clearly determined. It is thought that oxidative damage contributes to the process. The aim of this study was to determine the levels of coenzyme Q10 (CoQ10), DNA damage, and lipid peroxidation in patients with hydatidiform mole. *Materials and Methods:* The authors studied the levels of CoQ10, 8-hydroxydeoxyguanosine (8-OHdG), malondialdehyde (MDA) by high-performance liquid chromatography (HPLC), and the activity of glutathione peroxidase (GPX) by spectrophotometric method in blood obtained from patients with a complete hydatidiform mole (n=29), healthy pregnant women (n=29), and healthy non-pregnant women (n=29). *Results:* The 8-OHdG/dG ratio (2.8148 ± 0.81592) and MDA (10.8341 ± 4.64875 μmol) were significantly higher in patients with complete hydatidiform mole, while the ubiquinol-10/ubiquinone-10 ratio (0.2107 ± 0.15675) and GPX activity (43.4606 ± 18.31694 mU/ml) were lower ($p < 0.001$). *Conclusion:* The authors suggest that both mitochondrial oxidative and oxidative DNA damage play important roles in the pathogenesis of complete hydatidiform mole. Therefore supplementation of CoQ10 prevents recurrent gestational trophoblastic disease.

Key words: Coenzyme Q10; Ubiquinol; DNA damage; Oxidative stress; Antioxidants; Hydatidiform mole.

Introduction

Gestational trophoblastic disease arises more frequently in Asia than in North America or Europe [1]. Complete hydatidiform mole, in most cases, usually arises when an ovum without maternal chromosomes is fertilized by one sperm that then duplicates its DNA, resulting in a 46XX androgenetic karyotype, in which all chromosomes are paternally derived [2].

Reactive oxygen species products can damage many biological molecules; they can change protein functions, damage DNA material and cause lipid peroxidation of cell membranes [3-4]. Oxidative stress may play a critical role in carcinogenesis [5]. Reactive oxygen species (ROS) cause a breakage sequence in the DNA and modifications to occur in the base [6]. One of these base modifications is 8-hydroxydeoxyguanosine (8-OHdG), which is the most common base modification resulting from DNA oxidation [7]. This product is a sensitive marker of DNA oxidation [8]. Malondialdehyde (MDA) is the most common product of lipid peroxidation [9]. Glutathione peroxidase (GPX) is an important antioxidant enzyme that reduces the level of hydrogen peroxide and lipid peroxidation [10].

Coenzyme Q10 (CoQ10) is an important inhibitor of oxidative damage [11]. The oxidized form of CoQ10 is called ubiquinone-10 while the reduced form of CoQ10 is known as ubiquinol-10. Ubiquinol-10 is the first antioxidant to be oxidized when low-density lipoproteins are exposed to oxidants [12]. Therefore, the ubiquinol-10/ubiquinone-10 ratio may be a sensitive marker for studying disturbances in the pro-oxidant-antioxidant balance in human blood [13-15]. In recent study has shown that MDA level, increased DNA damage rate (level of 8-OHdG), and oxide CoQ10 enzyme level decreased in the lung cancer patients [16].

To evaluate DNA oxidation, lipid peroxidation, and antioxidant capacity in hydatidiform mole patients, the authors studied the 8-OHdG/dG ratio, MDA, GPX activity, and ubiquinol-10/ ubiquinone-10 ratio.

Materials and Methods

Eighty-nine women aged between 20 and 35 years were recruited from the Obstetrics and Gynecology Department of Yuzuncu Yil University Research Hospital, Van, Turkey. Of these, 29 had complete hydatidiform mole (group 1); 29 were healthy women in the first trimester of pregnancy with a single viable fetus (group 2) and 29 were healthy non-pregnant women, recruited as the control group (group 3). Recruitment was achieved via the women's own physicians and was conducted according to the process approved by the Human Ethical Review Board. Written informed consent was obtained from all subjects.

None of the patients was using any drugs at the time of blood sample collection. Venous blood samples were drawn from each patient during the 10th -19th week of gestation, following an

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Table 1. — *Characteristics of the complete hydatidiform mole (CHM), healthy pregnant (HP), and non-pregnant (NP) groups.*

	Group 1 (Mean±SD)	Group 2 (Mean±SD)	Group 3 (Mean±SD)	*p* value
Age	27.75±8.13	27.87±6.17	27.96±7.82	0.994
Gravidity	4.51±3.34	3.54±2.18	1.86±2.6	0.001
Parity	3.56±1.81	2.54±1.13	1.99±0.89	0.107
Gestational age (weeks)	10.93±1.70	11.28±1.16	0	0.000*

* Values are mean ± SE;
Different letters represent significant differences $p < 0.001$.

Table 2. — *The levels of 8-OHdG/dG, MDA, ubiquinol-10/ubiquinone-10, and GPX in the complete hydatidiform mole (CHM), healthy pregnant (HP), and non-pregnant (NP) groups.*

	Group1 (n=29) (Mean±SD)	Group2 (n=29) (Mean±SD)	Group 3 (n=29) (Mean±SD)
8-OHdG/dG	2.8148±0.81592*	1.6741±0.82216*	1.0849±0.53713*
MDA µL	10.8341±4.64875*	3.5576±1.32031*	3.8928±1.38162*
Ubiquinol-10/ ubiquinone-10	0.2107±0.15675*	2.6163±1.415*	4.0223±3.49895*
GPX mU/ml	43.4606±18.31694*	80.9767±35.97434*	105.514±97.1308*

*Values are mean ± SE;
Different letters represent significant differences $p < 0.001$.

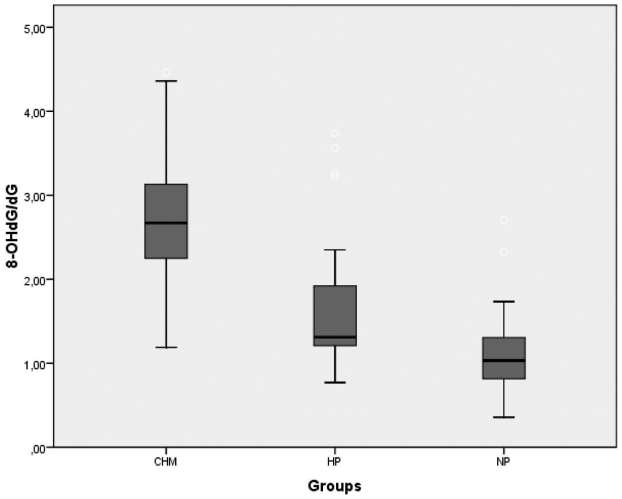

Figure 1. — 8-OHdG/dG levels in leukocyte DNA in patients complete hydatidiform mole (CHM), healthy pregnant (HP), and non-pregnant (NP) groups.

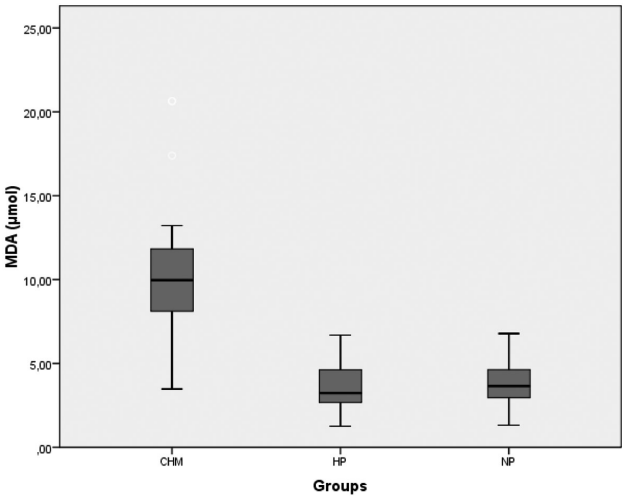

Figure 2. — MDA levels in patients complete hydatidiform mole (CHM), healthy pregnant (HP), and non-pregnant (NP) groups.

overnight fasting period. Sera were separated by centrifugation and the samples were processed immediately. The serum samples were placed in deionized polyethylene tubes and kept in a deep freeze at -20°C (without thawing) until the study day.

DNA isolation from blood was performed according to Miller *et al.* [17, 18] with some modifications. Two ml of blood with ethylene diamine tetraacetic acid (EDTA) was mixed with three ml of erythrocyte lysis buffer, incubated for ten minutes in ice, and then centrifuged (ten minutes at 3,500 rpm). The supernatant was decanted, and the pellet was thoroughly re-suspended in sodium dodecyl sulfate (10%, v/v), proteinase K (20 mg/ml), and 1.9 ml leukocyte lysis buffer. This mixture was then incubated at 65°C for one hour and mixed with 0.8 ml of 9.5 M ammonium acetate. After centrifugation at 3,500 rpm for 25 minutes, the clear supernatant (two ml) was transferred to a new sterile tube, and DNA was precipitated by addition of four ml of ice-cold absolute ethanol. DNA samples were dissolved in Tris EDTA buffer (ten mM, pH 7.4), and then hydrolyzed according to Shinenaga *et al.* method [19].

In the hydrolyzed DNA samples, 8-OHdG and dG levels were measured respectively by high-performance liquid chromatography (HPLC) with electrochemical (HPLC-ECD) and variable wavelength detector (HPLC-UV) systems, as was previously described [20]. CoQ10 and MDA levels were measured by a well-recognized HPLC method [21-22]. GPX activity was performed as described by Paglia and Valentine [23].

Statistical analyses were performed with SPSS (version 11.5). The statistical significance was calculated using ANOVA. A *p*-value of less than 0.05 was considered statistically significant. All the results were expressed as mean scores with their standard deviation (mean ± SD).

Results

Demographic data are shown in Table 1. The difference the authors observed between groups was statistically meaningful ($p < 0.001$). In group 1, the 8-OHdG/dG ratio (2.8148 ± 0.81592) and MDA (10.8341 ± 4.64875 µmol) values were statistically higher than in the others ($p < 0.001$) (Figures 1, 2). The ubiqunol-10/ubiquinone-10 ratio (0.2107 ± 0.15675) and GPX activity (43.4606 ± 18.31694 mU/ml) were lower with respect to the other two groups ($p < 0.001$) (Figures 3, 4) In group 2, while 8-OHdG/dG and ubiqunol-10/ubiquinone-10 levels were 1.6741 ±.0.82216 and 2.6163

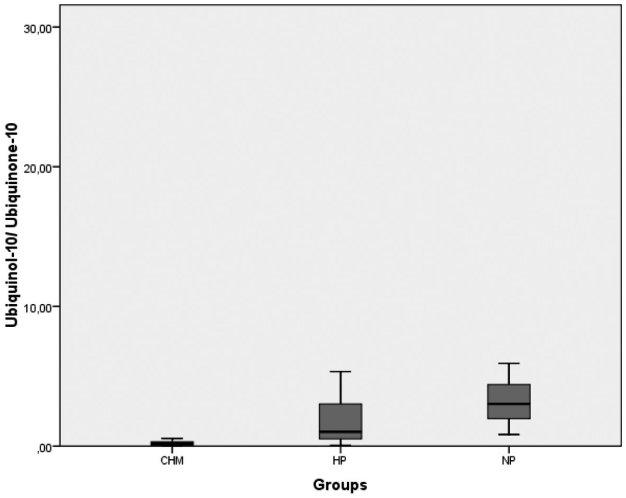

Figure 3. — Ubiquinol-10/ubiquinone-10 levels in patients complete hydatidiform mole (CHM), healthy pregnant (HP) and non-pregnant (NP) groups.

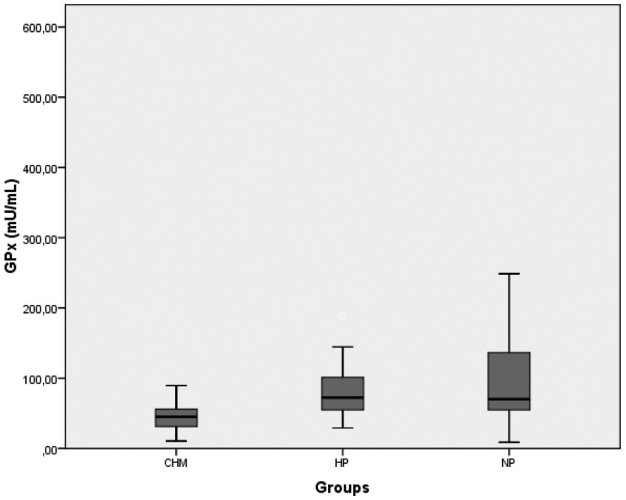

Figure 4. — GPX activity in patients complete hydatidiform mole (CHM), healthy pregnant (HP) and non-pregnant (NP) groups.

± 1.415, respectively. The MDA level and GPX activity were 3.5576 ± 1.32031 and 80.9767 ± 35.97434, respectively. All the group results are shown in Table 2.

Discussion

Oxidative stress plays a role in multiple physiological processes, from oocyte maturation to fertilization and embryo development. Oxidative stress can damage sperm DNA at different extents within an ejaculate and this could gradually affect the development of embryos [24]. The supplementation CoQ10 could improve the developmental competence of embryos produced in vitro and their ability to implant and give rise to healthy newborns [25]. There is evidence that oxidative stress is responsible for such conditions as abortion, pre-eclampsia, hydatidiform mole, fetal embryopathy, preterm labor, pre-eclampsia, and gestational diabetes, which lead to an immense burden of maternal and fetal morbidity and mortality [2]. ROS, derived from exogen and endogen sources, cause oxidative DNA damage, and this is related to mutagenous and carcinogenous formations [26]. Of the oxidative damage lesions, 8-OHdG is one of the most important mutagenic lesions and is a biomarker of oxidative DNA damage. In this study, the authors evaluated the DNA oxidation levels, lipid peroxidation, and antioxidant capacity among women who either had a complete hydatidiform mole or were healthy pregnant and non-pregnant. In the present study, the authors found that oxidative DNA damage was higher in patients with complete hydatidiform mole when compared with healthy pregnant and non-pregnant groups. This result was similar to that of another study, where levels of endogenous DNA damage in peripheral blood lymphocytes, determined by comet assay, were found increased in patients with complete hydatidiform mole [27].

In the present study, levels of oxidation products in mole hydatidiform pregnant group were found to be statistically higher than those found in the other two groups studied. 8-OHdG is repaired by the DNA glucosylase/AP lyase OGG1 enzyme. This enzyme cuts and removes oxidated bases on DNA, thus creating a defense mechanism. Since DNA is present in the nucleus, it has a more effectual defense mechanism than other biomolecules. In this study, it has been shown that oxidative damage provokes DNA augments in patients with complete hydatidiform mole. Furthermore, to fully understand its etiology, it is necessary to determine the activity of glucosylase/AP lyase OGG1 enzyme or polymorphisms in genes, which express this enzyme.

CoQ10 plays a crucial role in cellular metabolism, acting as an electron carrier between complexes I and II and complex III of the mitochondrial respiratory chain, and regulates uncoupling proteins, the transition pore, β-oxidation of fatty acids, and the nucleotide pathway [28]. CoQ10 deficiency has been linked with a variety of human disorders, some of which are caused by a direct defect of CoQ10 biosynthesis genes and some as a secondary event [29]. CoQ10 is a known modulator of gene expression [30-31] and inflammatory processes [32]. Because the reduced form of CoQ10, ubiquinol, is a potent antioxidant that protects lipids, DNA and proteins from oxidative damage [33, 34] ubiquinol supplementation might improve oxidative stress on protein and gene expression levels [35].

In the present study, the authors analyzed CoQ10 levels in patients with complete hydatidiform mole by calculating the ubiquinol-10/ubiquinone-10 ratio to determine CoQ10 levels. They observed that CoQ10 levels were statistically lower in a group of patients with complete hyda-

tidiform mole than those in healthy pregnant and non-pregnant groups. Moreover, they also found lower levels of CoQ10 in their healthy pregnant group patients than in their non-pregnant group. Harma *et al.* demonstrated that patients with complete hydatidiform mole have significantly higher levels of protein carbonyls and lower (but non-significant) levels of thiols, which are markers of oxidative status and lipid peroxidation [36]. MDA is a lipid peroxidation marker. In the present study, higher MDA concentrations were detected in patients with complete hydatidiform mole. In another study, the total peroxide and oxidative stress index increased in patients with complete hydatidiform mole [37]. Significantly lower mean levels of plasma total antioxidant response and significantly higher plasma total peroxide, oxidative stress index, and endogenous DNA damage were found in patients with complete hydatidiform mole compared with both pregnant and non-pregnant healthy women. Levels of oxidative damage to DNA were found to parallel the decrease in plasma total antioxidant response in all groups. This suggests that there is a link between the increased levels of oxidative stress and the increase in endogenous DNA damage seen in patients with complete hydatidiform mole compared with healthy pregnant women [27].

GPX is an antioxidant enzyme composed of a selenium atom. The present authors found that GPX activity significantly decreased in patients with complete hydatidiform mole compared to healthy pregnant and non-pregnant groups. In their previous study, they reported that catalase activity decreased in patients with complete hydatidiform mole [38]. In the same study, they also reported that vitamin A, D, and E levels were lower in complete hydatidiform mole patients than in healthy pregnant and non-pregnant groups [38]. These results may have significant implications in our understanding of the etiology of complete hydatidiform mole, since the decrease in GPX activity, one of the antioxidant system enzymes, and the increase in the level of MDA observed indicate that antioxidant-oxidant balance is disrupted in patients with complete hydatidiform mole. It is believed that a decrease in the level of CoQ10, which undertakes the role of electron carrier in mitochondrial metabolism, could be key in determining the etiology of complete hydatidiform mole. In conclusion, the authors suggest that offering CoQ10 supplementation to patients with complete hydatidiform mole may have a beneficial effect.

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