Catalase activity, serum trace element and heavy metal concentrations, vitamin A, vitamin D and vitamin E levels in hydatidiform mole

A. Kolusari[1], E. Adali[1], M. Kurdoglu[1], R. Yildizhan[1], A. Cebi[2], T. Edirne[3], H. Demir[4], I.H. Yoruk[4]

[1]*Department of Obstetrics and Gynaecology,* [2]*Department of Medical Biology,* [3]*Department of Family Medicine, Faculty of Medicine* [4]*Department of Chemistry, Faculty of Art and Science, Yuzuncu Yil University, Van (Turkey)*

Summary

Purpose of investigation: In this study we aimed to measure the activity of catalase, which is an antioxidant enzyme, the concentrations of some trace elements and heavy metals, and vitamin A, D and E levels in serum samples of patients with hydatidiform mole, normal pregnancies and healthy non pregnant women. *Methods:* Seventy-two women were enrolled in this study. Of these, 24 were healthy women in the first trimester of pregnancy (HP), 24 were healthy non-pregnant women (NP) and 24 were patients with complete hydatidiform mole (CHM). *Results:* Serum levels of catalase, Zn, Co, vitamin A, D and E were significantly lower in the CHM group when compared with the HP and NP groups ($p < 0.001$). Serum levels of Cu, Fe, and Cd were significantly higher in the CHM group when compared with the HP and NP groups ($p < 0.001$). *Conclusion:* The assessment of oxidant/antioxidant imbalance in pregnant women could be useful in the early determination of molar pregnancy and supplementation with antioxidants may be useful in the treatment of CHM, and may prevent recurrent molar pregnancy.

Key words: Catalase activity; Heavy metal; Trace element; Vitamin levels; Hydatidiform mole.

Introduction

Hydatidiform mole (HM) is a disorder of fertilization. It is characterized by a conceptus of hyperplastic trophoblastic tissue attached to the placenta [1]. Broad variations in the distribution of gestational trophoblastic disease (GTD) exist worldwide, with higher frequencies in some parts of Asia, the Middle East and Africa, with Turkey ranking in the middle of these countries [2]. The etiology of molar pregnancy is not completely understood [3]. Preeclamptic patients are exposed to increased oxidative stress [4, 5]. Placental hypersecretion of lipid peroxides or decreased placental antioxidant enzyme production may lead to endothelial dysfunction [6]. Placental abnormality and associated metabolic changes cause increased oxidative stress [7]. Similar metabolic changes are present in molar pregnancy [8]. The antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase (GPx) are synthesized in the body. Catalase is an effective scavenger of aqueous peroxide radicals [9]. The maternal blood levels of vitamin C, vitamin E, vitamin A, and β-carotene which has a strong provitamin A activity are decreased [10, 11]. Vitamin D might exert subtle oxidative stress, which could stimulate the detoxification mechanisms to protect cells from the subsequent stress challenges [12]. The studies on the roles of trace elements in health and disease over the past 50 years have led to a good understanding of their mode of action and why they are essential for life [13]. Some metals are essential to maintain the metabolism of the human body, whereas they can lead to poisoning because they tend to bioaccumulate [14]. Lead (Pb) and cadmium (Cd) increase oxidative stress [15].

In this study, we aimed to measure the activity of catalase which is an antioxidant enzyme; the concentrations of some trace elements and heavy metals (Cu, Zn, Cd, Co and Fe); vitamin A (retinol), vitamin D (cholecalciferol) and vitamin E (α-tocopherol) levels in serum samples of patients with hydatidiform mole, with normal pregnancies and healthy non-pregnant women.

Material and Methods

Seventy-two women were enrolled in this study. Of these, 24 were healthy women in the first trimester of pregnancy with a single viable fetus (HP). Twenty-four healthy non-pregnant women also participated as controls (NP). The remaining 24 subjects had complete hydatidiform mole (CHM). Written informed consent was obtained from all subjects.

None of these patients were using any drugs at the time of collection of the blood samples. Venous blood samples were drawn from each patient during the 10^{th}-19^{th} week of gestation after a fasting overnight period. Serum was 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 -20ºC (without thawing) until the study day.

Biochemical analysis of catalase activity in erythrocytes was determined according to the method defined by Aebi, in short, the supernatant (0.1 ml) was added to a quartz cuvette containing 2.95 ml of 19 mmol l^{-1} H_2O_2 solution prepared in potassium phosphate buffer (0.05M, pH 7.00) [16]. The change in absorbance was monitored at 240 nm over a 5-min period using a spectrophotometer (Shimadzu UV-1201, Japan). Determination of serum concentrations of Cu, Zn, Co, Cd, and Fe was performed by atomic absorption measurements, in which a

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UNICAM-929 spectrophotometer (Unicam Ltd, York Street, Cambridge, UK) was used. In addition, vitamin E, vitamin D and vitamin A levels were determined by a high-performance liquid chromatography (HPLC) method [17]. Data were expressed as mean ± standard error (SE) and analyzed using one-way repeated measures of analysis of variance (ANOVA). Duncan's test was used to test for differences among means for which one-way ANOVA indicated a significant ($p < 0.01$) F ratio.

Results

Demographic data of the subjects are shown in Table 1. There were no differences in mean age, gestational age, gravidity and parity between patients with CHM and HP ($p > 0.05$).

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

	CHM group (n = 24) Mean (SE)	HP group (n = 24) Mean (SE)	NP group (n = 24) Mean (SE)	*p* value
Age (years)	28.88 ± 1.71	29.17 ± 1.11	28.83 ± 0.87	NS
Gestational age (weeks)	15.71 ± 040	15.33 ± 0.56	–	NS
Gravidity	4.46 ± 0.74	4.00 ± 0.49	–	NS
Parity	3.13 ± 0.67	2.67 ± 0.50	–	NS

NS: nonsignificant.

Serum levels of catalase, vitamin A, D and E were significantly lower in the CHM group when compared with the HP and NP groups ($p < 0.001$). The mean values of serum levels of catalase, vitamin A, vitamin D, and vitamin E are given in Table 2.

Table 2. — *Serum levels of catalase and vitamins (A, D, E) of complete hydatidiform mole (CHM), healthy pregnant (HP), and non-pregnant (NP) groups.*

	CHM group (n = 24) Mean (SE)	HP group (n = 24) Mean (SE)	NP group (n = 24) Mean (SE)
Catalase EU/(gHb)$^{-1}$	3.01 ± 0.33^{a}	10.22 ± 1.12^{b}	11.86 ± 1.61^{b}
Vitamin A (mmol/l)	0.50 ± 0.04^{a}	0.72 ± 0.02^{b}	0.74 ± 0.03^{b}
Vitamin D (mmol/l)	0.02 ± 0.00^{a}	0.03 ± 0.00^{b}	0.03 ± 0.00^{b}
Vitamin E (mmol/l)	4.72 ± 0.34^{a}	6.26 ± 0.32^{b}	6.36 ± 0.20^{b}

Values are means ± SE; different letters represent significant differences ($p < 0.01$).

Serum levels of Cu, Fe, and Cd was significantly higher in the CHM group when compared with the HP and NP groups ($p < 0.001$). Serum levels of Zn and Co were statistically lower in the CHM group compared with the HP and NP groups ($p < 0.001$). Mean values of serum levels of Cu, Zn, Fe, Co and Cd are given in Table 3.

Table 3. — *Serum levels of Cu, Zn, Fe, Cd and Co of complete hydatidiform mole (CHM), healthy pregnant (HP), and non-pregnant (NP) groups.*

	CHM group (n = 24) Mean (SE)	HP group (n = 24) Mean (SE)	NP group (n = 24) Mean (SE)
Cu (µg/dl)	3.49 ± 0.16^{a}	2.82 ± 0.09^{b}	1.81 ± 0.08^{c}
Zn (µg/dl)	1.25 ± 0.08^{a}	1.71 ± 0.07^{b}	1.88 ± 0.09^{c}
Fe (µg/dl)	4.48 ± 0.71^{a}	1.79 ± 0.32^{b}	1.30 ± 0.07^{c}
Cd (µg/dl)	0.26 ± 0.03^{a}	0.02 ± 0.00^{b}	0.03 ± 0.00^{c}
Co (µg/dl)	0.03 ± 0.00^{a}	0.26 ± 0.03^{b}	0.24 ± 0.04^{c}

Values are means ± SE; different letters represent significant differences ($p < 0.01$).

Discussion

Reactive oxygen species (ROS) are continuously produced during normal physiologic events, and removed by antioxidant defence mechanisms. In pathological conditions, ROS are over produced and result in lipid peroxidation and oxidative damage [18, 19]. There is increasing evidence that oxidative stress is an important contributing factor in the pathogenesis of preeclampsia [9]. Similar increasing oxidative stress is present in molar pregnancy [8]. We found that catalase was significantly lower in the CHM group when compared with the HP and NP groups. The importance of vitamin E and β-carotene in preventing free radical damage is well known and their levels have been reported to be significantly decreased in preeclampsia [11]. Maternal vitamin D deficiency may be an independent risk factor for preeclampsia. Vitamin D supplementation in early pregnancy should be explored for preventing preeclampsia and promoting neonatal well-being [12]. There is no study evaluating vitamin A, D and E levels in hydatidiform moles in the English literature. In the present study, we observed significantly lower levels of vitamin A, vitamin D and vitamin E in CHM patients when compared with the HP and NP groups. Significant changes in serum trace metal concentrations, particularly zinc and copper, have been documented during normal pregnancies. Harma *et al.* reported that levels of zinc in serum were found to be significantly higher and levels of copper in serum were significantly lower in hydatidiform mole patients than controls [20]. On the contrary, Wang *et al.* reported that the contents of Cu in erythrocytes were found to be significantly higher and the contents of Zn significantly lower in patients with hydatidiform mole than those in normal females [21]. In our study, we found that zinc was significantly lower and copper was significantly higher in the CHM group when compared with the HP and NP groups.

Some metals are essential to maintain the metabolism of the human body, whereas they can lead to poisoning because they tend to bioaccumulate [14]. The Industrial Revolution, which increased opportunities of occupational and environmental exposure to metals among women, revealed their adverse effects on pregnancy [22]. Lead and cadmium are established toxic and carcinogenic metals [23]. Lead and cadmium increase oxidative stress [15]. Iron is a redox-active transition metal and can participate in single electron reactions and catalyse formation of free radicals, including the undesirable hydroxyl radicals [24, 25]. There is no study evaluating heavy metal levels in hydatidiform moles in the literature. Serdar *et al.* observed a significant increase in serum iron levels in mild and severe preeclampsia patients when compared to normotensive healthy women [26]. In our study we found that cadmium and iron were significantly higher and cobalt was significantly lower in the CHM group when compared with the HP and NP groups.

The assessment of oxidant/antioxidant imbalance in pregnant women could be useful in the early determination of molar pregnancy. Supplementation with antioxidants in pregnant women with low antioxidant status may be useful in the treatment of CHM and may prevent recurrent molar pregnancy. However, more studies are needed to verify and clarify the relationship between antioxidant levels and heavy metal levels and hydatidiform mole.

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Address reprint requests to:
A. KOLUSARI, M.D.
Yuzuncu Yil Universitesi
Arastirma Hastanesi
Kadin Hastaliklari ve Dogum Anabilim Dali
Van (Turkey)
e-mail: dralikolusari@yahoo.com