Role of plasma nitric oxide in complete hydatidiform mole

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Summary

Purpose of investigation: This prospective study aimed to evaluate any relationship between development of complete hydatidiform mole and plasma levels of nitric oxide (a biologically active mediator derived from L-arginine), and human chorionic gonadotropin β (β-hCG; a metabolite involved in trophoblast production).

Methods: Levels of plasma nitric oxide and β-hCG were measured in 38 patients with complete hydatidiform mole pregnancies, and nitric oxide levels were measured in 31 women with normal pregnancies who formed the control group.

Results: For patients compared with controls, mean plasma concentrations of nitric oxide were significantly higher (35.84 vs 29.54 μM; $p < 0.001$) and significantly associated with increased risk of hydatidiform mole (odds ratio 1.0105, 95% confidence interval 1.0034-1.0176). No significant relationship was found between plasma levels of nitric oxide and β-hCG in the patient group.

Conclusion: In patients with complete hydatidiform mole compared with controls, plasma nitric oxide levels were found to be significantly higher and associated with increased molar risk.

Key words: hCG; Hydatidiform mole; Nitric oxide.

Introduction

Gestational trophoblastic disease is a heterogeneous group of diseases characterized by abnormally proliferating trophoblastic tissues; it includes partial and complete hydatidiform mole (CHM), invasive mole, choriocarcinoma and placental site trophoblastic tumour [1].

Nitric oxide (NO) is a product of the conversion of L-arginine to L-cytrulline by nitric oxide synthase [2]. Since its identification in 1987 [3], an exponential amount of research has identified NO as an important mediator in physiological and pathological processes [4, 5]. Human studies have investigated activity of NO synthase in pregnancy [6] and trophoblastic diseases [7]. Ramsay *et al.* measured nitric oxide synthase activity in myometrium and trophoblasts throughout gestation [6]. The highest levels of nitric oxide synthase activity were found in first trimester villi, with a significant fall in activity by the third trimester [6]. More recently, there have been reports identifying isoforms of nitric oxide synthase present in trophoblast cells [8]. For example, Ariel et al. have shown immunoreactivity of an endothelial nitric oxide synthase in early gestation and in trophoblastic disease, and have suggested that this enzyme may play a role in implantation and vascular invasion [5].

Plasma levels of human chorionic gonadotropin β (β-hCG), a metabolite involved in trophoblast production, are proportional to the number of trophoblastic cells present in blood. Patients with CHM commonly have markedly elevated plasma β-hCG levels prior to evacuation. After molar evacuation, clinical measurement of plasma β-hCG is carried out routinely and continues for six months to confirm remission.

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The present study sought to determine any relationship between levels of plasma nitric oxide, β-hCG, and hydatidiform mole.

Materials and Methods

Sixty-nine patients in the first trimester of pregnancy, attending Harran University Hospital between July 1998 and September 2002, participated in this prospective case-control study. Of these, 31 normal pregnant healthy women served as controls and 38 patients were diagnosed with CHM. All controls had a single viable fetus, mean gestational age 13.2 weeks as estimated by ultrasonography. Patients suffering from CHM had a mean gestational age of 12.9 weeks according to the last menstrual period (p not significant). Demographic characteristics of the study groups are shown in Table 1.

Admission criteria for the patient group were absence of the following factors: previous cardiovascular disease, diabetes mellitus, renal disease, primary hypertension, connective tissue disease, history of antioxidant intake, and medication. Diagnosis of trophoblastic disease was based on histopathologic examination.

Blood Sampling. Blood samples of all patients were taken before the evacuation. After an overnight fast, blood (4 ml) was drawn from the antecubital vein using a Vacutainer tube (BD Vacutainer Systems, Oxford, U.K.). The collected blood was centrifuged at 1000 g for ten minutes. Plasma was separated and stored in aliquots at –80°C until needed for determination of nitric oxide and β-hCG levels.

Nitric Oxide Assay. Plasma nitric oxide levels (mM) were determined using a commercial kit (Nitric Oxide Colorimetric Assay; Roche, Germany) and Automatic Tektime MicroElisa equipment (Organon Tekniko; Microwell Systems, the Netherlands).

β-hCG Assay. Plasma β-hCG levels were determined by a chemiluminescence method using an automatic hormone analyzer (Immulite 2000; EURO/DPC, Gwynedd, U.K.). Values are expressed as mIU/ml.

Statistical Analysis. All statistical analyses were performed using SPSS for Windows Version 11.0. Pearson correlation analysis was used for comparison of nitric oxide and β-hCG levels. Logistic regression was used for analysis of nitric oxide values with regard to risk of development of hydatidiform mole. The Student's t-test was used for comparison of mean values of the demographic variables (age, gestational age, gravidity, parity, abortions and live children); $p \leq 0.05$ was considered statistically significant.

Results

For patients with CHM, mean ± SD plasma concentrations of nitric oxide were found to be significantly higher than controls (35.84 ± 7.88 vs 29.54 ± 7.94 μM; $p < 0.001$) (Table 1).

In patients with CHM, the mean ± SD plasma β-hCG levels were 262,200 ± 403,530 mIU/ml (range: 174,000-2,500,000 mIU/ml). No correlation was found between values obtained for nitric oxide and β-hCG levels in plasma.

Logistic regression analysis was used to evaluate the risk interaction between plasma nitric oxide levels and CHM. Higher levels of nitric oxide were found to increase CHM risk significantly (OR = 1.0105, CI = 1.0034-1.0176).

Table 1. — *Demographic characteristics of patients.*

	Mean ± SD		
	Complete hydatidiform mole (n = 38)	Controls (n = 31)	p
Age (years)	31.0 ± 8.3	28.6 ± 5.4	0.173
Gravidity	4.1 ± 2.0	3.1 ± 2.1	0.060
Parity	2.6 ± 1.8	1.8 ± 1.7	0.081
Abortion	0.4 ± 0.7	0.2 ± 0.6	0.252
Live children	2.5 ± 1.7	1.8 ± 1.7	0.098
Gestational age (weeks)	12.9 ± 4.8	13.2 ± 4.4	0.704

Mean ± SD plasma concentrations of nitric oxide in patients with complete hydatidiform mole and in healthy pregnant control patients.

Discussion

Nitric oxide is involved in the regulation of endocrine functions, but only a few studies have reported its role in placental hormone secretion. Myat et al. investigated the function of nitric oxide in release of hCG in the two different choriocarcinoma cell lines JEG-3 and BeWo. Findings in their study supported the hypothesis that nitric oxide produced in these cell lines is involved in the regulation of hCG secretion [9].

Quantitative measurement of plasma levels of β-hCG is important in both diagnosis and follow-up of trophoblastic disease because β-hCG levels are known to be proportional to the number of trophoblastic cells present [10]. Patients with CHM commonly have markedly elevated hCG levels pre-evacuation. Menczer et al. reported that 30 (41%) of 74 patients with CHM had pre-evacuation hCG values greater than 100,000 mIU/ml [11]. Similarly, Genest et al. noted that 46% of 153 patients with CHM, managed at the New England Trophoblastic Disease Center between 1980 and 1990, had pre-evacuation hCG levels above 100,000 mIU/ml. Markedly elevated circulating hCG values are suggestive of a diagnosis of CHM [12]. In our study, mean hCG levels in plasma of CHM patients were also greater than 100,000 mIU/ml. However, the results did not show any significant relationship between hCG levels (which reflect trophoblast number) and nitric oxide levels in plasma.

Enzyme nitric oxide synthase was originally characterized in choriocarcinoma cells by Western blot analysis [9]. Nitric oxide synthase activity has been observed in solid human tumour tissues, including gynecological cancer [13] and breast cancer [14]. Moriyama *et al.* suggested that the increased plasma nitrite/nitrate levels correlated with tumour volume in patients with hepatocellular carcinoma [15]. All women in the present study were in the first trimester of pregnancy, where nitric oxide synthase activity is reported to be high [4]. However, results of the present study showed a significantly increased level of plasma nitric oxide in CHM patients compared with normal pregnant women. The clinical significance of this even more elevated circulating nitric oxide associated with hydatidiform mole is not clear. Ariel et al. have suggested that nitric oxide release by trophoblastic cells may play a role during vascular invasion in placentation and in trophoblastic disease [5].

Conclusion

This case-control study examined the association between plasma concentrations of nitric oxide and risk of CHM. To the best of our knowledge, this is the first study reporting such an association in humans. Significantly higher mean plasma levels of nitric oxide were found in patients with CHM compared with healthy pregnant patients of the same gestational age. Although no significant relationship was observed between plasma levels of nitric oxide and β-hCG levels, elevated plasma nitric oxide levels were observed as a significant risk factor of CHM. Further studies are needed to understand the significance of increased plasma nitric oxide levels in patients with CHM.

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