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Research Article

Association between the 8-OHdG Level in Placental/Umbilical Cord Blood and Maternal/Neonatal Characteristics at Full-Term Birth

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Abstract

Objective

The aim of this study was to estimate the relationship between the levels of a DNA damage biomarker, 8-hydroxy-deoxyguanosine (8-OHdG), in placental/umbilical cord blood (CB) and maternal/neonatal characteristics at full-term birth.

Methods

We used ELISA kits to measure the 8-OHdG levels in CB in mothers with full-term normal vaginal deliveries. The possible relationships between the 8-OHdG levels and infant birth weight, placental weight and placental weight per infant birth weight were assessed.

Results

The 8-OHdG levels in the CB ranged from 0.11 to 1.19 ng/ml, with a median of 0.41 ng/ml (mean: 0.43 ± 0.21 n/ml). Significant positive correlations were observed between the 8-OHdG levels and placental weight or placental weight per infant birth weight (r=0.343, p=0.007, r=0.368, p=0.004, respectively). However, no significant correlations were observed between the 8-OHdG levels and infant birth weight. In addition, when the maternal body mass index (BMI) values were classified into three groups, significant positive correlations were observed between the 8-OHdG levels and placental weight per infant birth weight in the BMI 18.5–25 group and between the 8-OHdG levels and placental weight in the BMI ≥ 25 group.

Conclusions

These findings demonstrate the possibility that the 8-OHdG level in CB is correlated with placental growth and suggest that the maternal physique affects this correlation.

Keywords: Oxidative Stress; 8-OHdG; Placental/Umbilical Cord Blood; Body Mass Index; Placenta

Introduction

Oxidative stress is defined as a disturbance in the prooxidant/antioxidant balance in favor of the former, leading to potential damage [1]. Oxygen is one of the most important elements required to sustain life. However, the oxidizing potential of oxygen can result in toxic biological effects. Some of the oxygen taken up by the body during respiration is used to generate reactive oxygen species (ROS) during energy metabolism or defense against pathogens. ROS may induce oxidative damage to DNA and are associated with various diseases, including cancer, diabetes, metabolic syndrome, hypertension and atherosclerosis [2-6].

Previous studies have suggested that the levels of one of the typical biomarkers of DNA damage, 8-hydroxy-deoxyguanosine (8-OHdG), are increased in patients with bladder carcinoma, prostate cancer [7], childhood cancers [8,9], diabetes mellitus [10,11], coronary heart disease [12] and myoma uteri [13], as well as in smokers [14-16]. Moreover, the 8-OHdG levels are higher in males than females [17,18]. The concentrations of lipid peroxidation products in the peripheral blood are generally higher in pregnant women than in non-pregnant women because the rapidly growing fetoplacental unit requires a large amount of oxygen in utero [19-21]. In addition, pregnancy-induced hypertension is associated with maternal oxidative stress [19, 22-24].

As the unborn child grows in the uterus, it receives all of its necessary nutrition and oxygen requirements from the mother through the placenta. Immediately after birth, the newborn baby must adapt from a hypoxic environment (PO_2 of 20–25 torr) to a high oxygen environment (PO_2 of 100 torr) and is readily affected by oxidative stress [25]. Recent reports have suggested associations between fetal oxidative damage and fetal growth restriction [26] and between birth weight and gestational age [27]. However, there is little information regarding the relationship between the state of oxidative stress in the newborn and placental weight. We recently reported that the 8-OHdG levels in placental/umbilical cord blood (CB) collected from a smoking group were significantly higher than those observed in CB collected from a non-smoking group [28].

In the present study, we evaluated the relationships between the levels of a DNA damage biomarker, 8-hydroxy-deoxyguanosine (8-OHdG), in CB and maternal/neonatal growth characteristics at full-term birth.

Materials and Methods

Subjects and CB Sample Collection

Between August 2010 and February 2012, 60 CB units were collected at a single hospital (Hirosaki National Hospital, Hirosaki, Japan) after obtaining informed consent from all mothers and approval from the Committee of Medical Ethics of Hirosaki National Hospital (Hirosaki, Japan) and the Committee of Medical Ethics of Hirosaki University Graduate School of Medicine (Hirosaki, Japan). The inclusion criteria were a singleton birth, full-term gestation, vaginal delivery and birth without resuscitation or the use of immediate rescue procedures. In the present study, only a non-smoking group of mothers was assessed in order to exclude the effects of smoking. A segment of the umbilical cord was double-clamped immediately after neonatal delivery, and blood was obtained from the umbilical vein before placental delivery (i.e., in utero collection). The CB was collected into a sterile collection bag containing 28 mL of citrate phosphate dextrose anticoagulant (CBC-20; Nipro, Osaka, Japan) immediately after delivery. The serum was separated within 24 hours of CB collection. Eppendorf tubes filled with the separated serum were stored at −80°C until the analysis of biochemical markers. Relevant perinatal data, including maternal age, maternal weight, maternal body mass index (BMI), smoking status, gestational age, duration of labor, birth weight, placental weight, Apgar score and the umbilical artery acid/base status and gas levels were obtained from labor and delivery records .

Quantitative Analysis of the 8-OHdG Levels

The 8-OHdG levels in the CB were measured using highly sensitive 8-OhdG ELISA kits (Jaica, Fukuroi, Japan). Each assay was performed immediately after thawing the serum sample. To remove high-molecular-weight proteins, which interfere with this analysis, each CB serum sample was filtered through an ultrafiltration membrane (molecular weight cutoff: 10,000; Amicon Ultra, Tokyo, Japan). The filtrate was concentrated to one-fifth of the initial volume by using a SpeedVac® centrifugal evaporator (Thermo Scientific Savant SPD1010; Thermo Fisher Scientific, Suwanee, GA, USA).

Statistical Analysis

To determine the involvement of the maternal condition, the placental weight per Kg of infant birth weight was calculated. In addition, in order to identify differences related to maternal physique, the study population consisted of 60 pregnant females who were classified into three groups: underweight (BMI < 18.5), normal weight (BMI 18.5–24.9) and obese (BMI ≥ 25) according to the BMI based on the criteria of the World Health Organization. In the present study, the overweight (BMI 25–29.9) and obese (BMI > 30) groups were combined to form a BMI ≥ 25.0 obese group. The statistical analysis was performed using the SPSS version 21.0 (SPSS Japan, Inc., Tokyo, Japan) and Origin (Origin Lab, Northampton, MA, USA) for Windows software packages. The results are presented as medians (ranges) and percentages. Associations between variables were assessed using Spearman's rank correlation coefficient. Categorical variables were compared using the Chi-square test. A *P*-value of <0.05 was considered to be significant.

Results

Study Population Characteristics

The characteristics of our study population are summarized in Table 1. The maternal age ranged from 18 to 43 years, with a median of 31 years; 53.3% of the mothers were primiparas. The prepregnancy BMI values ranged from 16.5 to 45.8 Kg/m^2, with a median of 20.9 Kg/m^2. Taking into consideration the wide BMI range, the mothers were classified into three groups according to the maternal BMI. The proportion of mothers in the BMI < 18.5, BMI 18.5–24.9 and BMI ≥ 25 groups was 16.7%, 70.7% and 13.3%, respectively. The birth weights ranged from 2,502 to 4,304 g, with a median value of 3,176 g. A total of 58.3% of the infants were male, and the median gestational age at birth was 39 weeks. The placental weights ranged from 385 to 850 g, with a median value of 555 g. All newborns were delivered vaginally, 18.3% were treated with maternal oxygen administration during labor and 6.7% underwent vacuum extraction. The CB 8-OHdG levels ranged from 0.11 to 1.19 ng/ml, with a median of 0.41 ng/ml (mean: 0.43 ± 0.21 ng/ml). In the study population, there were pregnancy complications, such as pregnancy-induced hypertension or gestational diabetes mellitus. In addition, none of the mothers consumed alcohol during pregnancy.

Table 1. Study Population Characteristics.

	Median (range)	
Maternal characteristics		
Maternal age (years)	31	(18–43)
Prepregnancy BMI (Kg/m^2) [a]	20.9	(16.5–45.8)
< 18.5 (%) [c]	16.7	(16.7)
18.5 – 24.9 (%) [c]	70.0	(70.0)
≥ 25.0 (%) [c]	13.3	(13.3)
Gestational weight gain (Kg) [b]	8.6	(–4.3–14.8)
Alcohol use [c]	0	(0)
Parity		
Primipara [c]	32	(53.3)
Multipara [c]	28	(46.7)
First stage of labor (min)	395	(30–2520)
Second stage of labor (min)	22	(1–191)
Oxygen administration [c]	11	(18.3)
Vacuum extraction	4	(6.7)
Neonatal factors		
Gestational age (weeks)	39	(37–41)
Birth weight (g)	3176	(2502–4304)
Placental weight (g)	555	(385–850)
Neonatal sex		
Male [c]	35	(58.3)
Femal[e] c	25	(41.7)
Apgar score		
1 min	9	(8–9)
5 min	9	(9–10)
CB		
Umbilical artery pH	7.31	(7.19–7.45)
8-OHdG (ng/mL)	0.41	(0.11–1.19)

[a] BMI based on the self-reported prepregnancy weight. [b] Gestational weight gain based on the ratio of self-reported prepregnancy weight to that at delivery. [c] The values are numbers and percentages. n=60.

Correlations between the 8-OHdG Levels and Maternal/Neonatal Characteristics

We assessed the possible correlations between the 8-OHdG levels and maternal/neonatal characteristics. The CB 8-OHdG levels were positively correlated with the placental weights (r = 0.343; Fig. 1A). A more significant positive correlation was observed between the 8-OHdG levels and the placental weights per infant birth weight (r = 0.368; Figure. 1B). Meanwhile, no significant correlations were found between the 8-OHdG levels and the other maternal/neonatal factors, including the umbilical artery pH values (data not shown). In this study population, 11 mothers were placed on oxygen therapy during labor; their CB 8-OHdG levels ranged from 0.13 to 0.75 ng/ml. However, there were no significant differences between the group treated with oxygen inhalation (0.40 ng/ml (0.11–1.19)) and the group treated without oxygen inhalation (0.44 ng/ml (0.13–0.75)).

Table 2. Correlations between the 8-OHdG Level and infant/placental weight.

	8-OHdG		
BMI	< 18.5 [a]	18.5 – 24.9 [b]	≥ 25.0 [c]
Birth weight	-0.115	-0.121	0.690
Placental weight	0.580	0.229	0.778*
Placental weight (g)/ Infant birth weight (Kg)	0.539	0.344*	0.190

Spearman's rank correlation coefficient: *P < 0.05.

[a] n=10, [b] n=42, [c] n=8.

The correlation between the maternal BMI values and 8-OHdG levels was analyzed using Spearman's rank correlation coefficient (Table 2). Consequently, the levels of 8-OHdG in the BMI 18.5–25 group were significantly positively correlated with the placental weights per infant birth weight. Similarly, the 8-OHdG levels were correlated with the placental weights in the BMI ≥ 25 group.

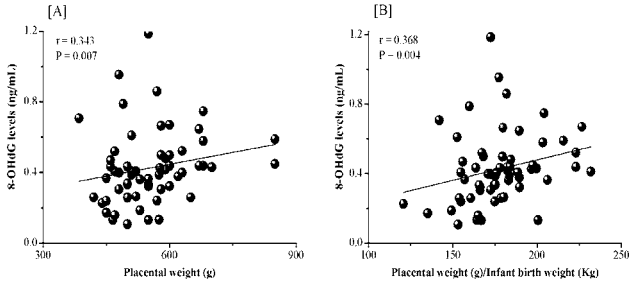

Figure 1. Correlations between the CB 8-OHdG level and placental weight (**A**) and placental weight per infant birth weight (**B**) (n = 60). There were significant positive correlations between these variables.

Discussion

The CB 8-OHdG levels in the new-borns with singleton vaginal deliveries ranged from 0.11 to 1.19 ng/ml (Table 1). Forlenza et al. reported that the serum 8-OHdG levels are much lower than the urine 8-OHdG levels. In their study, the serum 8-OHdG levels were approximately 0.20–1.26 ng/ml in healthy adults [29]. The values obtained in the present study were approximately equal to the levels detected in healthy adults. Additionally, Schulpis et al. compared the 8-OHdG levels in CB between new-borns with vaginal and cesarean section deliveries [30,31] and found that the mean 8-OHdG levels were 0.25 ng/mL and 0.27 ng/mL, respectively, which indicates that there are no significant differences between delivery modes. In the present study, no significant associations were observed between these factors. Because no comparisons were made between the 8-OHdG levels in maternal blood and those in CB in the present study, is not possible to draw a definitive conclusion based only on the present data.

The present results showed that the 8-OHdG levels increased in association with the placental weight in the maternal BMI ≥ 25 group (Table 2, Figure.1). The placenta is the primary source of maternal oxidative stress during normal pregnancy, and increased lipid peroxidation is a normal phenomenon in pregnancy [32]. In addition, obesity during pregnancy is considered to be a high-risk state because it is associated with many complications. Fujimaki et al. reported that oxidative stress may also contribute to the development of FGR [26]. Meanwhile, the placental tissue is not the only source of ROS, as pregnancy is a pro-inflammatory state associated with an increased number of granulocytes and, consequently, a decreased number of lymphocytes and monocytes [32]. Additionally, the expression of adhesion molecules on the cell surface of circulating leukocytes indicates the activation of leukocytes, resulting in the release of cytokines, which may lead to the generation of superoxide and other ROS as part of the immune response [33]. Considering the above points, the present results suggest the possibility that excessive growth of the placenta associated with maternal obesity increases the oxidative stress of the fetus. However, since the present study was conducted based on hospital records, we were unable to obtain full information regarding the mothers' diets (nutritional status), including food and beverages, affecting the fetal environment. Therefore, it was not possible to identify the relationships between maternal lifestyle-related factors and the 8-OHdG levels in CB. More precise approaches are required to clarify the relationships between the 8-OHdG levels and maternal obesity.

More than 95% of the oxygen taken in during breathing is ultimately converted into water or reduced via hydroxylation reactions by drugs. The remaining fraction of oxygen is not returned to the environmental air, thus leading to the formation of ROS. Essentially, an unborn child is exposed to an extremely low oxygen environment. Meanwhile, oxygen is essential for fetal development, and hypoplasia and placental dysfunction may delay fetal growth. In cases involving a high ratio of placental weight per infant birth weight, oxygen is readily supplied from the mother; thus, large amounts of ROS may be generated. Although further studies should be performed, the correlation shown in Figure 1 suggests the possibility that ROS produced by the placenta contribute to the generation of 8-OHdG, depending on factors of the maternal physique, such as obesity and BMI.

The relationships among the CB 8-OHdG levels, oxidative stress and unborn child growth/development have not yet been sufficiently studied. It has been demonstrated that the intrauterine environment has an effect on the development of future diseases in the child. Therefore, determining the state of intrauterine oxidative stress and its effects on the unborn child is extremely important for disease prevention. More precise approaches are required to elucidate the relationships between the external environment, maternal lifestyle factors and fetal oxidative stress.

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