Original Article

Pain-reducing anesthesia prevents oxidative stress in human term placenta

Yoko Tsuzuki,[1,2,3] Yoriko Yamashita,[1,3,*] Yuka Hattori,[1,4] Guang Hua Li,[1] Shinya Akatsuka,[1] Tomomi Kotani,[4] Fumitaka Kikkawa,[4] Aya Naiki-Ito,[3] Satoru Takahashi,[3] Kimitoshi Nishiwaki[2] and Shinya Toyokuni[1,*]

[1]Department of Pathology and Biological Responses, [2]Department of Anesthesiology and Resuscitation and [4]Department of Obstetrics and Gynecology, Nagoya University Graduate School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya 466-8550, Japan
[3]Department of Experimental Pathology and Tumor Biology, Nagoya City University Graduate School of Medical Sciences, 1 Kawasumi, Mizuho-cho, Mizuho-ku, Nagoya 467-8601, Japan

(Received 31 October, 2015; Accepted 12 November, 2015; Published online 17 February, 2016)

Anesthesia is sometimes used for the reduction of maternal pain in normal human term labor, but whether the drugs affect oxidative stress remains unclear. The placenta serves as an interface between the maternal and fetal vasculature. In this study, we immunohistochemically analyzed two markers for oxidative stress, namely 8-hydroxy-2'-deoxyguanosine (8-OHdG) and 4-hydroxy-2-nonenal–modified proteins (HNE), using placentas from 21 cases of normal tansvaginal delivery (V group), 20 Caesarean sections (C group), and 17 normal transvaginal deliveries with epidural anesthesia (E group). 8-OHdG staining in the nuclei of trophoblasts lining the chorionic villi was significantly stronger in the V group either compared with the C or E group ($p<0.001$), without significant differences in the C and E groups ($p = 0.792$). Moderate to intense staining by HNE of the intravascular serum of chorionic villi vasculature was frequently observed in the placentas from the V group, but less frequently of those in either C or E groups ($p<0.001$), nor the p value comparing the C and E groups was significant ($p = 0.128$) for HNE staining. Our results suggest that although the role of oxidative stress and its influences on fetal state in the placenta in labor remains unclear, it seems to be lessened by epidural anesthesia.

Key Words: **oxidative stress, placenta, anesthesia, 8-hydroxy-2'-deoxyguanosine, 4-hydroxy-2-nonenal-modified proteins**

Epidural anesthesia is widely used not only for Caesarean section but also for normal transvaginal labour and delivery.[1] The relationship between oxidative stress and pregnancy has been widely studied, and a certain amount of reactive oxygen species (ROS) is necessary for the normal development of human embryos and fetuses, but an excess of ROS or oxidative stress may cause placental dysfunction, which may affect fetal development.[2] Whether maternal anesthesia has any effect on the generation of placental oxidative stress is mostly unknown.

8-Hydroxy-2'-deoxyguanosine (8-OHdG) is a frequently used marker for evaluating oxidative stress.[3] Nuclear immunostaining can be observed in various conditions associated with oxidative stress, such as in the human epidermis in association with aging and sun-exposure and in rat hepatic ischemia-reperfusion or myocardial infarction.[4–6] In human pregnancy, 8-OHdG staining has been observed in the placentas of pre-eclampsia patients with elevated serum uric acid,[7] and the serum concentration of 8-OHdG has been significantly associated with prolonged labor.[8]

4-Hydroxy-2-nonenal-modified proteins (HNE) is another marker for oxidative stress.[9,10] Elevated HNE levels have been reported in the sera of type 2 diabetes mellitus patients,[11] skin-flaps after ischemia, hepatic ischemia-reperfusion rat models,[5,12] human alcoholic liver disease,[13] and chorioamnionitis.[14]

In this study, we performed immunohistochemical analysis to evaluate whether any correlation exists between 8-OHdG and HNE levels and the usage of epidural anesthesia in the human placenta.

Materials and Methods

Placental samples. Placentas were obtained from 21 cases of normal transvaginal delivery (V group), 20 Caesarean sections (C group), and 17 normal transvaginal deliveries with epidural anesthesia (E group). The clinical details of the mothers and babies are summarized in Table 1. Written permission was obtained from each mother. The ethical committees of Nagoya University Graduate School of Medicine, Bell Research Center for Reproductive Health and Cancer, and Asamoto Clinic approved the experiments. The placentas were immediately fixed with phosphate-buffered saline neutralized 10% formalin, cut within 24 h, and then further fixed for a total of 24 to 48 h. Formalin-fixed, paraffin-embedded sections were used for evaluation; they were obtained using a transverse section from the maternal to fetal surface at the intermediate area from the umbilical cord to the terminal villi of the placenta.

Immunohistochemistry. Immunohistochemistry for the detection of 8-OHdG and HNE was performed as previously described[15,16] using primary antibody clones, N45.1[3] and HNE-J2,[9,17] respectively. For a positive control, 6-week-old male Wistar rats were subjected to intraperitoneal injection of 15 mg of iron/kg of ferric nitrotriacetate (Fe-NTA) which was prepared immediately before use, as previously described.[3,9] The animals were sacrificed 1 h after injection. Formalin-fixed, paraffin-embedded kidney samples from Fe-NTA-treated or untreated rats were used to optimize the immunostaining procedure. Dilutions of the primary antibodies and the methods for antigen retrieval were ×2,000 and proteinase K digestion for 8-OHdG and ×10,000 and heat addition for HNE. Immunostaining was performed using a Bond-III automatic immunohistochemistry staining system (Leica, Wetzlar, Germany). Positive staining for 8-OHdG was scored using a Keyence BZ-9000 microscope (Keyence, Osaka, Japan) by automatically scoring the intensity of positive nuclear staining for 20 chorionic villi for each placenta, using a cut-off value of 70 for the signal intensity. The analysis was performed in triplicate. For HNE staining, the degree of positively stained vasculature was compared in low-magnification images from 3 sections.

*To whom correspondence should be addressed.
E-mail: k46581a@nucc.cc.nagoya-u.ac.jp (YY); toyokuni@med.nagoya-u.ac.jp (ST)

doi: 10.3164/jcbn.15-138

Table 1. Clinical data of the 58 placentas

Sample no.	Maternal age	Birth weight (g)	Gender of baby	Apgar score	Duration of labor (h:min)	Anesthetics	Complication	Multipara or primi-gravida
V-1	37	3,234	male	9	9:37	—	n.p.	p
V-2	31	3,404	male	8	22:27	—	n.p.	p
V-3	33	2,486	female	9	3:40	—	n.p.	p
V-4	35	2,648	female	9	7:43	—	n.p.	p
V-5	29	3,296	male	9	4:58	—	n.p.	p
V-6	31	3,248	male	8	12:46	—	n.p.	p
V-7	22	2,980	female		6:01	—	n.p.	p
V-8	26	2,790	female	9	11:39	—	n.p.	p
V-9	—	—	—	—	—	—	stillborn (34 weeks)	p
V-10	27	3,128	male	9	15:33	—	n.p.	p
V-11	35	3,040	female	9	14:09	—	n.p.	p
V-12	24	3,048	female	9	5:12	—	n.p.	p
V-13	24	3,082	male	9	21:43	—	n.p.	p
V-14	33	3,034	female	9	4:20	—	n.p.	p
V-15	28	2,682	male	9	15:10	—	n.p.	p
V-16	35	2,880	male	9	10:12	—	n.p.	p
V-17	30	3,284	female	9	17:45	—	n.p.	p
V-18	35	3,484	female	9	3:17	—	n.p.	p
V-19	24	3,246	female	9	15:50	—	n.p.	p
V-20	29	3,130	male	10	27:30	—	n.p.	p
V-21	31	3,480	female	9	7:15	—	n.p.	p
C-1	30	2,942	male	9	—	0.5% bupivacaine 2.6 ml	n.p.	NK
C-2	31	3,268	male	9	—	propofol 100 mg, fentanyl 80 mg, pentazocine 30 mg, 1% sevoflurane 7 min, N_2O 40l 32 min	n.p.	NK
C-3	30	2,960	male	9	—	0.5% bupivacaine 2.3 ml	n.p.	p
C-4	34	3,578	male	9	—	0.5% bupivacaine 2.5 ml	n.p.	p
C-5	29	3,326	male	9	—	0.5% bupivacaine 2.5 ml	n.p.	p
C-6	36	3,608	male	9	—	0.5% bupivacaine 2.6 ml	n.p.	p
C-7	25	2,536	male	9	—	0.5% bupivacaine 2.5 ml, fentanyl 0.4 ml	n.p.	p
C-8	29	2,980	male	9	—	0.5% bupivacaine 2.5 ml	n.p.	p
C-9	32	3,488	male	8	—	0.5% bupivacaine 2.5 ml	n.p.	p
C-10	33	3,098	male	10	—	0.5% bupivacaine 2.5 ml	n.p.	p
C-11	36	3,310	male	8	—	0.5% bupivacaine 2.3 ml	n.p.	p
C-12	31	2,950	male	10	—	0.5% bupivacaine 2.5 ml	n.p.	p
C-13	25	2,524	male	5	—	0.5% bupivacaine 2.5 ml × 2	n.p.	p
C-14	33	3,284	female	9	—	0.5% bupivacaine 2.2 ml	n.p.	p
C-15	26	2,940	male	8	—	0.5% bupivacaine 2.3 ml	n.p.	p
C-16	31	2,818	female	8	—	0.5% bupivacaine 2.5 ml	n.p.	p
C-17	26	2,940	male	8	—	0.5% bupivacaine 2.2 ml	n.p.	p
C-18	33	3,366	male	7	—	0.5% bupivacaine 2.2 ml	n.p.	p
C-19	28	2,986	female	9	—	0.5% bupivacaine 2.5 ml	n.p.	p
C-20	27	2,706	female	10	—	0.5% bupivacaine 2.2 ml	n.p.	p
E-1	34	3,210	male	9	6:32	0.25% levobupivacaine hydorochloride 10 ml	n.p.	mu
E-2	27	2,980	male	9	9:55	0.25% levobupivacaine hydorochloride 10 ml	n.p.	p
E-3	25	3,040	male	8	7:16	0.25% levobupivacaine hydorochloride 10 ml	n.p.	p
E-4	36	3,125	male	9	6:06	0.25% levobupivacaine hydorochloride 10 ml	n.p.	p
E-5	37	3,880	male	9	12:40	0.25% levobupivacaine hydorochloride 10 ml	n.p.	mu
E-6	28	2,770	male	9	4:20	0.25% levobupivacaine hydorochloride 10 ml	cervical cancer	p
E-7	33	3,230	male	9	3:08	0.25% levobupivacaine hydorochloride 10 ml	n.p.	mu
E-8	31	2,835	male	9	6:50	0.25% levobupivacaine hydorochloride 10 ml	n.p.	p
E-9	35	3,515	male	8	6:16	0.25% levobupivacaine hydorochloride 10 ml	n.p.	p
E-10	34	3,010	female	8	7:45	0.25% levobupivacaine hydorochloride 10 ml	n.p.	p
E-11	28	3,030	female	9	16:55	0.25% levobupivacaine hydorochloride 10 ml	n.p.	p
E-12	35	3,215	male	9	2:52	0.25% levobupivacaine hydorochloride 10 ml	n.p.	mu
E-13	32	3,545	female	9	13:20	0.25% levobupivacaine hydorochloride 10 ml	n.p.	p
E-14	35	3,265	female	9	7:07	0.25% levobupivacaine hydorochloride 10 ml	n.p.	p
E-15	29	3,105	male	9	16:10	0.25% levobupivacaine hydorochloride 10 ml	n.p.	p
E-16	30	3,130	female	8	2:05	0.25% levobupivacaine hydorochloride 10 ml	hyperthyroidism	mu
E-17	28	3,050	female	8	6:16	0.25% levobupivacaine hydorochloride 10 ml	n.p.	p

V group, normal transvaginal delivery; C group, Caeserean section group; E group, normal transvaginal delivery with anesthesia. n.p., nothing particular; p, primigravida; mu, multipara; NK, not known.

Statistical analysis. The results were analysed using analysis of variance and Student's t test. $P<0.05$ was considered to be statistically significant.

Results

Clinical data. The clinical data of the 3 groups are summarized in Table 1. The durations of labor varied from 3 h 17 min to 27 h 23 min (median 10 h 55 min) in the V group and from 2 h 5 min to 16 h 55 min (median 6 h 50 min) in the E group. The ages of the mothers and the weights of the babies did not differ among the 3 groups.

Placental histology. All of the human placentas included in this study were found to have normal term development, and none of the cases showed chorioamnionitis or tumors. The size of the placentas, number of syncytial knots, and percentage of infarcted areas did not differ among the groups. Representative results of H&E staining and immunohistochemical staining for the analysis of oxidative stress are shown in Fig. 1.

8-Hydroxy-2'-deooxyguanosine (8-OHdG). Positive 8-OHdG immunostaining was observed in the nuclei of the syncytiotrophoblasts at the surfaces of the chorionic villi of the placentas from the V group (Fig. 1A). In contrast, the levels of 8-OHdG immunostaining were significantly lower in the placentas from both the C group and the E group (Fig. 1B and C). Significant differences were observed between the V group and the C group ($p<0.001$) and the V group and the E group ($p<0.001$), but not between the C group and the E group ($p = 0.792$) (Fig. 2).

4-Hydroxy-2-noneal-modified proteins (HNE). Positive HNE immunostaining was frequently observed in the vessel lumens in the chorionic villi in the V group (Fig. 1D). In contrast, the frequency of positive staining was significantly lower in the placentas from both the C group and the E group (Fig. 1E and F). Significant differences were observed between the V group and the C group ($p<0.001$) and between the V group and the E group ($p<0.001$), but not between the C group and the E group ($p = 0.128$) (Fig. 2).

Discussion

This is the first study to report the relationship between pain-reducing anesthesia and oxidative stress in human placenta. According to a previous report, anesthesia and analgesia do not affect the health state of the newborn, as indicated by Apgar score; cord blood pH; the occurrence of hypoglycemia, hyperbilirubinemia, and respiratory depression; the lack of changes in the levels of cortisol, beta-endorphin, and two oxidative stress markers (total hydroperoxide and advanced oxidation protein products) in the cord arterial blood and extremely high levels of 2 cytokines (IL-1 beta and IL-8) in the epidural analgesia group.[18] Although numerous previous studies have examined the association between pregnancy and oxidative stress, evidence that maternal anesthesia affects the condition of the fetus or neonate is lacking.[19] While a certain amount of reactive oxygen species (ROS) is necessary for normal embryonic and fetal development, an excess of ROS or oxidative stress is associated with maternal obesity, smoking and hypertension. Furthermore, excess ROS affects the placental microvasculature and, therefore, has negative effects on fetal health in regards to fetal intrauterine growth retardation and maternal pre-eclampsia.[2,20–22] ROS are small

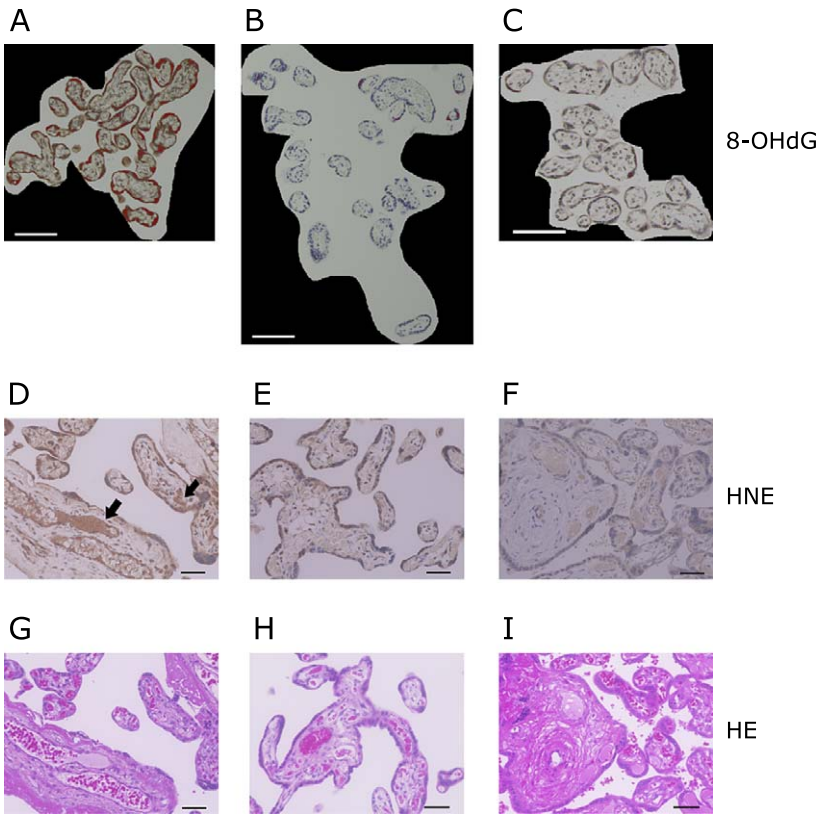

Fig. 1. Immunostaining results of 20 chorionic villi stained with 8-hydroxy-2'-deoxyguanosine (8-OHdG) are shown for the V group (A), C group (B), and E group (C). Signal intensities greater than 70 are highlighted in red; this was done by the BZ9000 analysis software. Many red signals are observed in the V group compared to in the C group and E group. The results of immunostaining for 4-hydroxy-2-nonenal-modified protein (HNE) are shown for the V group (D), C group (E) and E group (F). Note that some cross sections of the vessels are positive in the chorionic villi of the V group (arrows). Hematoxylin and eosin (H&E) staining of representative sections of placentas from the V group (normal transvaginal delivery) (G), C group (Caesarean section) (H), and E group (transvaginal delivery with epidural anesthesia) (I).

doi: 10.3164/jcbn.15-138

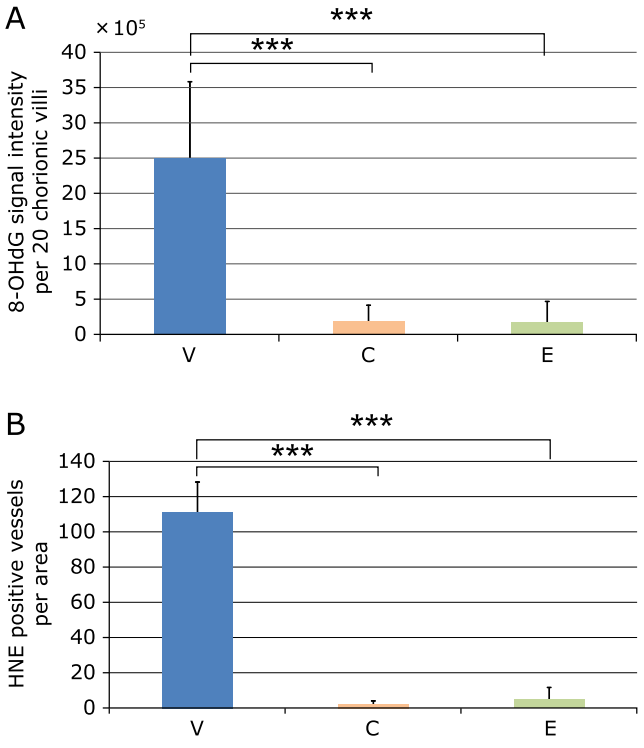

Fig. 2. (A) Signal intensity of immunopositivity for 8-hydroxy-2'-deoxyguanosine (8-OHdG) for the V (normal transvaginal delivery), C (Caesarean section), and E (transvaginal delivery with epidural anesthesia) groups. (B) Number of positive vessels per low-magnification area identified by 4-hydroxy-2-nonenal–modified proteins (HNE) immunostaining for the 3 groups. ***p<0.001.

molecules that can easily be transferred through maternal-fetal interfaces; thus, the presence of oxidative stress markers in the placenta should reflect maternal oxidative stress that may negatively affect the fetus. This study showed for the first time that oxidative stress is reduced by maternal anesthesia as indicated by the levels of 2 different markers of oxidative stress.

In our analysis, 8-OHdG staining was mostly observed in the nuclei of viable syncytiotrophoblasts lining the chorionic villi. 8-OHdG is a marker of oxidized DNA caused by either acute or chronic oxidative stress,[23–25] and elevated 8-OHdG and HNE are observed in Fe-NTA-treated mouse kidneys as early as 1 h post peritoneal injection.[23] Thus, the positive staining of these 2 markers in our study could reflect acute production of ROS that was somehow caused by the maternal condition during labor or during the surgical procedure in the case of Caesarean section. Positive staining for 8-OHdG was also observed in the endothelial cells of the vessels in the chorionic villi and apoptotic syncytiotrophoblastic cells and syncytial knots of the V group placentas. Further analyses are necessary to clarify the biological implications of these findings because positive staining in viable versus non-viable syncytiotrophoblasts may differ, and oxidative stress may cause effects that disrupt the term placenta in a manner that is not related to the maternal condition. The reason for the presence of HNE in the vessels is also unclear. HNE is typically observed in the cytoplasm of cells undergoing lipid peroxidation,[15] but a previous report suggested that it is related to oxidized serum albumin level in diabetes mellitus patients,[11] thus, positive HNE staining of the vessel lumens in our study may also reflect oxidation of serum proteins. Alternatively, the observed positive HNE staining may have indicated the presence of peroxidized lipids in the vasculature, and the direct target of HNE staining in this study remains unclear.

In this study, we analyzed placentas from mothers who delivered by three distinct methods: normal transvaginal delivery, Caesarean section, and normal transvaginal delivery with epidural

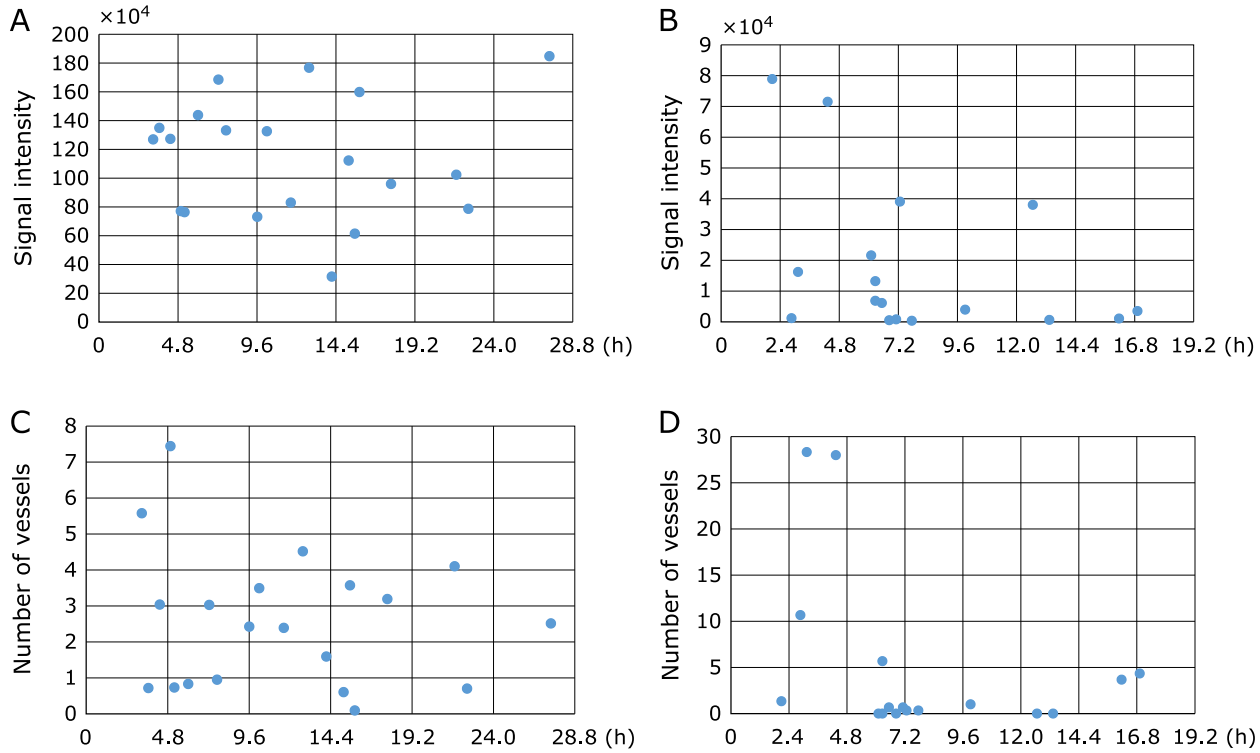

Fig. 3. Labor duration and oxidative stress. Labor time (h: hours) and signal intensity of immunopositivity for 8-hydroxy-2'-deoxyguanosine (8-OHdG) for the V (normal transvaginal delivery) group (A) and E (transvaginal delivery with epidural anesthesia) group (B); number of positive vessels identified by 4-hydroxy-2-nonenal–modified protein (HNE) immunostaining for the V group (C), and E group (D).

analgesia for pain reduction. Previous studies have most commonly divided methods of delivery into 2 groups, normal transvaginal and Caesarean section, when focusing on the relationship between labor and oxidative stress.[8,26–28] With the exception of one study,[26] oxidative stress levels have been found to be lower in Caesarean section patients,[8,27,28] emphasizing that oxidative stress-induced defense systems are up-regulated during labor.[8,27] In this study, we included the E group to clarify whether labor or anesthesia contributes to changes in oxidative stress levels, and we succeeded in demonstrating that anesthesia may have positive effects on reducing oxidative stress in the term placenta for the first time. The median durations of labor in the V, E, and C groups were 10 h 55 min, 6 h 50 min, and 0 h 0 min, respectively. Therefore, we cannot exclude the possibility that a longer duration of labor contributed to higher levels of oxidative stress in the V group; however, the labor duration times in each group had no correlation with the signal intensity of 8-OHdG or the numbers of HNE-positive vessels (Fig. 3), and no significant difference was observed between the E and C groups, which had very different labor durations. Thus, we suggest that the observed differences in the oxidative stress markers were probably due to the administration of anesthesia to the mothers, in other words, whether the mothers experienced pain during delivery.

In conclusion, for the first time, we evaluated oxidative stress by immunostaining of human placenta for 8-OHdG and HNE following deliveries performed with or without maternal anesthesia. We successfully demonstrated that oxidative stress was significantly reduced in the placenta of mothers who delivered under epidural analgesia. Pain reduction may benefit both mothers and newborns.

Acknowledgments

The authors thank the mothers for donating the placentas used in this study. The authors also thank Mr. N. Misawa, Ms. J. Takekawa and Mr. K. Kato for excellent technical support. The authors are grateful for the members of Asamoto Clinic, Bell Clinic Group and also for the members of the ethnic committee of Aasamoto Clinic for cooperation.

Abbreviations

Fe-NTA	ferric nitrotriacetate
HNE	4-hydroxy-2-nonenal-modified proteins
8-OHdG	8-hydroxy-2'-deoxyguanosine
ROS	reactive oxygen species

Conflict of Interest

No potential conflicts of interest were disclosed.

References

1 Hawkins JL. Epidural analgesia for labor and delivery. *N Engl J Med* 2010; **362**: 1503–1510.
2 Dennery PA. Oxidative stress in development: nature or nurture? *Free Radic Biol Med* 2010; **49**: 1147–1151.
3 Toyokuni S, Tanaka T, Hattori Y, *et al*. Quantitative immunohistochemical determination of 8-hydroxy-2'-deoxyguanosine by a monoclonal antibody N45.1: its application to ferric nitrilotriacetate-induced renal carcinogenesis model. *Lab Invest* 1997; **76**: 365–374.
4 Toyokuni S, Hirao A, Wada T, *et al*. Age- and sun exposure-dependent differences in 8-hydroxy-2'-deoxyguanosine and N-(carboxymethyl)lysine in human epidermis. *J Clin Biochem Nutr* 2011; **49**: 121–124.
5 Yamagami K, Yamamoto Y, Kume M, *et al*. Formation of 8-hydroxy-2'-deoxyguanosine and 4-hydroxy-2-nonenal-modified proteins in rat liver after ischemia-reperfusion: distinct localization of the two oxidatively modified products. *Antioxid Redox Signal* 2000; **2**: 127–136.
6 Miwa S, Toyokuni S, Nishina T, *et al*. Spaciotemporal alteration of 8-hydroxy-2'-deoxyguanosine levels in cardiomyocytes after myocardial infarction in rats. *Free Radic Res* 2002; **36**: 853–858.
7 Fukushima K, Murata M, Tsukimori K, Eisuke K, Wake N. 8-Hydroxy-2-deoxyguanosine staining in placenta is associated with maternal serum uric acid levels and gestational age at diagnosis in pre-eclampsia. *Am J Hypertens* 2011; **24**: 829–834.
8 Schulpis KH, Lazaropoulou C, Vlachos GD, *et al*. Maternal-neonatal 8-hydroxy-deoxyguanosine serum concentrations as an index of DNA oxidation in association with the mode of labour and delivery. *Acta Obstet Gynecol Scand* 2007; **86**: 320–326.
9 Toyokuni S, Uchida K, Okamoto K, Hattori-Nakakuki Y, Hiai H, Stadtman ER. Formation of 4-hydroxy-2-nonenal-modified proteins in the renal proximal tubules of rats treated with a renal carcinogen, ferric nitrilotriacetate. *Proc Natl Acad Sci U S A* 1994; **91**: 2616–2620.
10 Toyokuni S, Miyake N, Hiai H, *et al*. The monoclonal antibody specific for the 4-hydroxy-2-nonenal histidine adduct. *FEBS Lett* 1995; **359**: 189–191.
11 Toyokuni S, Yamada S, Kashima M, *et al*. Serum 4-hydroxy-2-nonenal-modified albumin is elevated in patients with type 2 diabetes mellitus. *Antioxid Redox Signal* 2000; **2**: 681–685.
12 Um SC, Suzuki S, Toyokuni S, Uchida K, Hiai H, Nishimura Y. Formation of 4-hydroxy-2-nonenal-modified proteins and 3-nitro-L-tyrosine in rat island skin flaps during and after ischemia. *Ann Plast Surg* 1999; **42**: 293–298.
13 Ohhira M, Ohtake T, Matsumoto A, *et al*. Immunohistochemical detection of 4-hydroxy-2-nonenal-modified-protein adducts in human alcoholic liver diseases. *Alcohol Clin Exp Res* 1998; **22**: 145S–149S.
14 Temma K, Shimoya K, Zhang Q, *et al*. Effects of 4-hydroxy-2-nonenal, a marker of oxidative stress, on the cyclooxygenase-2 of human placenta in chorioamnionitis. *Mol Hum Reprod* 2004; **10**: 167–171.
15 Kobayashi H, Yamashita Y, Iwase A, *et al*. The ferroimmunomodulatory role of ectopic endometriotic stromal cells in ovarian endometriosis. *Fertil Steril* 2012; **98**: 415–422.e1–e12.
16 Yamashita Y, Nagasaka T, Naiki-Ito A, *et al*. Napsin A is a specific marker for ovarian clear cell adenocarcinoma. *Mod Pathol* 2015; **28**: 111–117.
17 Okazaki Y, Wang Y, Tanaka H, *et al*. Direct exposure of non-equilibrium atmospheric pressure plasma confers simultaneous oxidative and ultraviolet modifications in biomolecules. *J Clin Biochem Nutr* 2014; **55**: 207–215.
18 Dani C, Perugi S, Fontanelli G, *et al*. Effects of epidural and systemic maternal analgesia in term infants: the NoPiL study. *Front Biosci (Elite Ed)* 2010; **2**: 1514–1519.
19 Littleford J. Effects on the fetus and newborn of maternal analgesia and anesthesia: a review. *Can J Anaesth* 2004; **51**: 586–609.
20 Aycicek A, Varma M, Ahmet K, Abdurrahim K, Erel O. Maternal active or passive smoking causes oxidative stress in placental tissue. *Eur J Pediatr* 2011; **170**: 645–651.
21 Malti N, Merzouk H, Merzouk SA, *et al*. Oxidative stress and maternal obesity: feto-placental unit interaction. *Placenta* 2014; **35**: 411–416.
22 Hattori Y, Mukaide T, Jiang L, *et al*. Catalytic ferrous iron in amniotic fluid as a predictive marker of human maternal-fetal disorders. *J Clin Biochem Nutr* 2015; **56**: 57–63.
23 Tanaka T, Nishiyama Y, Okada K, *et al*. Induction and nuclear translocation of thioredoxin by oxidative damage in the mouse kidney: independence of tubular necrosis and sulfhydryl depletion. *Lab Invest* 1997; **77**: 145–155.
24 Jiang L, Zhong Y, Akatsuka S, *et al*. Deletion and single nucleotide substitution at G:C in the kidney of gpt delta transgenic mice after ferric nitrilotriacetate treatment. *Cancer Sci* 2006; **97**: 1159–1167.
25 Akatsuka S, Yamashita Y, Ohara H, *et al*. Fenton reaction induced cancer in wild type rats recapitulates genomic alterations observed in human cancer. *PLoS One* 2012; **7**: e43403.
26 Mutlu B, Aksoy N, Cakir H, Celik H, Erel O. The effects of the mode of delivery on oxidative-antioxidative balance. *J Matern Fetal Neonatal Med* 2011; **24**: 1367–1370.
27 Buhimschi IA, Buhimschi CS, Pupkin M, Weiner CP. Beneficial impact of term labor: nonenzymatic antioxidant reserve in the human fetus. *Am J Obstet Gynecol* 2003; **189**: 181–188.
28 Vakilian K, Ranjbar A, Zarganjfard A, *et al*. On the relation of oxidative stress in delivery mode in pregnant women; a toxicological concern. *Toxicol Mech Methods* 2009; **19**: 94–99.

doi: 10.3164/jcbn.15-138