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Changes in Maternal Lipid Peroxidation Levels and Antioxidant Enzymatic Activities Before and After Delivery

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Abstract

Objective: Our goal was to characterize the changes in maternal lipid peroxidation levels and antioxidant enzymatic activities before and after delivery.

Methods: Predelivery and 1,24, and 48 hours post-partum plasma concentrations of malondialdehyde, erythrocyte enzyme superoxide dismutase, glutathione peroxidase and catalase were measured in uncomplicated pregnancies.

Results: Malondialdehyde levels increased slightly from predelivery to 24 hours post-partum and then decreased significantly at 48 hours post-partum. At one hour post-partum superoxide dismutase and catalase levels increased significantly to about 125% and 170% of predelivery levels, respectively. Thereafter, these values decreased significantly from one hour to 48 hours post-partum. The relative changes in superoxide dismutase and catalase levels at one hour post-partum compared to predelivery values correlated significantly with the duration of labor.

Conclusion: The results suggest that the uncontrolled lipid peroxidation caused by reactive oxygen species, which are produced in consequence of tissue reoxygenation, may occur during labor and that prolonged labor, may cause maternal oxidative stress. (J Nippon Med Sch 2000; 67: 434—439)

Key words: maternal, lipid peroxidation, antioxidant enzymatic activities, delivery

Introduction

Lipid peroxidation is normal phenomenon that occurs continuously at low levels in all humans. These peroxidation reactions are in part toxic to cells and cell membranes; however, they are normally controlled by countervailing biologic mechanisms. Severe oxidative stress produces reactive oxygen free radicals and induces uncontrolled lipid peroxidation[1]. Since the cell membranes consist primarily of lipids, uncontrolled lipid peroxidation can cause cell injury and death via DNA strand breakage and membrane damage[2].

During pregnancy, oxidative stress has also been associated with reproductive problems. Many studies have found maternal lipid peroxide levels in blood to be significantly elevated in preeclampsia compared to normal pregnancy[3-8], with decreased antioxidant levels[3,8-10]. However, there is only scattered information about baseline levels in healthy pregnant women, particularly in post partum. Although several investigators have described and quantified the maternal and fetal response to the pain and stress of labor, there is

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no information about the relationship between lipid peroxidation and clinical characteristics.

It is known that oxygenation of both maternal and fetal tissue oscillate frequently during labor[11].Maternal oxygen consumption increases significantly in normal labor[12,13]. It is also reported that there are often periods of apnea and/or shallow respiration between uterine contractions[14]. In addition, the uterine contractions cause a reduction in uterine blood flow[15-17]. These evidences make it likely that uncontrolled lipid peroxidation caused by reactive oxygen species, which are produced in consequence of tissue reoxygenation, may occur during labor.

Therefore, the aim of the present study was to follow the time course of changes in maternal lipid peroxidation levels and in antioxidant enzymatic activities before and after delivery and to explore the relationship between lipid peroxidation and the clinical characteristics of labor.

Materials and Methods

Measurements of the plasma index of lipid peroxidation and erythrocyte antioxidant enzymatic activities were performed on 25 nulliparous subjects at Nippon Medical School Tama Nagayama Hospital. All of these subjects provided written informed consent for participation in this study, which was approved by the institutional review board. Demographic and clinical data were collected at routine obstetric visits. All of these subjects had uncomplicated singleton pregnancies. Liver, kidney, and thyroid functions were normal both before and during pregnancy. The women also denied any history of chronic disease, cigarette or illicit drug use. A first-trimester or early second-trimester ultrasonographic study confirmed gestational age.

Progress of labor was determined by vaginal examinations every one to two hours and as indicated by clinical conditions. Uterine contractions and fetal heart rate were monitored continuously with a cardiotocograph. None of these subjects showed any abnormalities during labor and delivered spontaneously.

Blood samples were obtained by venous puncture from the antecubital vein of each woman before delivery and 1, 24, and 48 hours post partum and were immediately transferred to chilled heparinized glass tubes. Predelivery samples were collected at 36 weeks of gestation. Samples were centrifuged at 1,000 g for 10 minutes, and plasma was divided into aliquots and frozen in dry ice prior to being stored at −80℃. Erythrocyte fractions were resuspended to the original blood volume and washed with cold isotonic saline solution. The erythrocytes were hemolyzed in distilled water and stored at −80℃ until analysis.

Malondialdehyde, a metabolite of lipid peroxides detectable in plasma, was used as an indicator of lipid peroxidation. Plasma malondialdehyde concentrations were estimated as reactive substances by a thiobarbituric acid adduction method described by Yagi[18] and Wang et al.[19]: the assay indirectly quantifies lipid hydroperoxides by measuring aldehyde breakdown products of lipid peroxidation. In summary, four milliliters of 1/12 sulfuric acid and 0.5 m*l* of 10% phosphotungstic acid were added to 20 μ*l* sample and mixed thoroughly. After centrifugation at 3,000 g for 10 minutes, the liquid phase was decanted. Four milliliters of double-distilled water and 1.0 m*l* thiobarbituric acid reagent (0.67% 2-thiobarbituric acid/acetic acid, 1: 1) were then added to each sample, mixed, and heated at 95℃ for 1 hour. Samples were cooled with tap water. Five milliliters of *n*-butyl-alcohol was added, and the samples were vigorously shaken for 1 minute and centrifuged. The *n*-butyl-alcohol phase, which contained the lipid peroxides, was used for malondialdehyde analysis with a fluorospectrophotometer (Shimazu RF-5,000, Tokyo, Japan) with excitation at 515 nm and emission at 553 nm. The individual performing the assay was blinded to the identity of the patient.

Erythrocyte enzymes superoxide dismutase, glutathione peroxidase and catalase were used as intracellular antioxidant markers. Superoxide dismutase and glutathione peroxidase activities were determined by spectrophotometric assay described respectively by Flohe and Otting (1984)[20] and Flohe and Gunzler (1984)[21], while catalase activity was measured using the first order rate constant (K) of decomposition of hydrogen peroxide[22]. All of these antioxidant enzymatic activities were expressed relative to the hemoglobin concentration[23].

All data were expressed as mean ± standard deviation. One-way analysis of variance followed by

Scheffés F test was used to compare the values within each marker of lipid peroxidation and antioxidant. Regression analysis was used to evaluate the relationships between these markers and clinical data. Differences with a P value of less than 0.05 were considered to be statistically significant.

Results

The characteristics of the subjects are shown in **Table 1**. Their gestational age, duration of labor and blood loss were within the normal range. None of the subjects showed secondary arrest of labor, which requires stimulation of uterine activity and active management of labor (e.g. forceps delivery and vacuum extraction), and abnormal hemorrhage, which requires blood transfusion. All of the newborn weights were appropriate for the gestational age. Their Apgar score at 1 and 5 minutes were within the normal range.

Table 1 Characteristics of subjects (n=25)

	Mean±SD	Range
Mother		
Age (y)	27.9±3.9	23 to 38
Gestational age at delivery (wks)	38.8±1.1	37 to 41
Duration of labor (h)	9.4±6	1 to 30
Blood loss during delivery (g)	204±163.1	60 to 630
Baby		
Weight (g)	3,014±367.6	2,510 to 3,535
1-minute Apgar score	9*	7 to 10
5-minute Apgar score	9*	7 to 10

Note. *Median value

As shown in **Table 2**, plasma concentration of malondialdehyde increased slightly from predelivery to 24 hours post partum and then decreased significantly at 48 hours post partum. One hour after delivery erythrocyte enzymes superoxide dismutase and catalase levels increased significantly to about 125% and 170% of predelivery levels, respectively. Thereafter, these values decreased significantly from one hour to 48 hours post partum. Changes in glutathione peroxidase activity were qualitatively similar to those in superoxide dismutase and catalase, but these changes did not reach significance.

The relative changes in superoxide dismutase and catalase levels at one hour post partum compared to predelivery values correlated significantly with the duration of labor (**Fig. 1**). However, malondialdehyde and glutathione peroxidase did not correlate significantly with any of the clinical parameters.

Discussion

The results of the present study demonstrate that the maternal lipid peroxide levels in blood increase slightly immediately after delivery with a marked increase in antioxidant levels. These changes in antioxidant levels correlated significantly with the duration of labor. Thereafter, the maternal lipid peroxide and the antioxidant levels decreased at 48 hours post partum.

Several studies have attempted to evaluate the ma-

Table 2 Changes in plasmatic lipoperoxidative index: Malondialdehyde (MDA) and erythrocytic antioxidant enzymatic superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) activities before and after delivery

	MDA nmol/ml	SOD μg/gHb	CAT K/gHb	GSH-Px IU/gHb
predelivery	2.30±0.30	138.6±31.2	71.1±32.9	21.6±4.9
1h post partum	2.31±0.26	175.6±44.2*	121.4±46.2*	23.1±5.6
24h post partum	2.51±0.43	173.2±52.2*	79.4±38.1†	24.5±4.9
48h post partum	1.91±0.31§	115.6±31.2†§	61.5±42.1†	18.0±4.8

Note: Values are mean±SD, *$p<0.05$ for the difference against predelivery (one factor ANOVA followed by Scheffé's post hoc test), †$p<0.05$ for the difference against one hour post partum (one factor ANOVA followed by Scheffé's post hoc test), §$p<0.05$ for the difference against 24 hours post partum (one factor ANOVA followed by Schefflé's post hoc test)

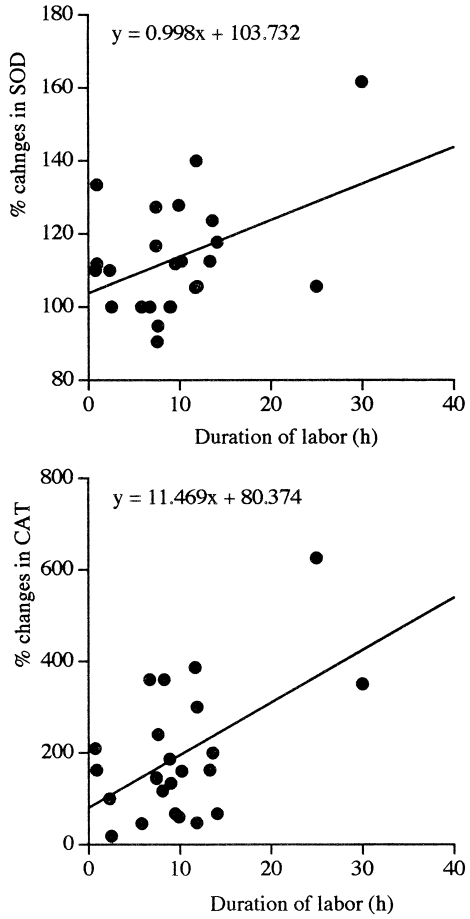

Fig. 1 Scattergram illustrates the correlation of relative changes in (a) superoxide dismutase (SOD) and (b) catalase (CAT) at one hour post partum and duration of labor. A significant relationship was found between the duration of labor and both SOD ($r=0.44$, $p<0.05$) and CAT ($r=0.51$, $p<0.05$).

ternal lipid peroxidation in healthy pregnant and non-pregnant women[19,24-29]. According to these studies, lipid peroxide levels in the first trimester of pregnancy were sometimes higher and sometimes lower than the level of the non pregnant control group. By the second trimester, increases of 10 to 50% over first trimester values were usually seen. Third trimester levels sometimes, but not always, declined. In the present study, maternal lipid peroxide levels in the third trimester are in good agreement with those reported by Wang et al.[19], who used the same technique as us to study maternal lipid peroxide levels throughout normal pregnancy. These levels, together with the present data, showed a moderate increase compared with those in non-pregnant controls[19,23,30].

The source of increased lipid peroxide during pregnancy is unknown. Increased levels may be related to the increase in serum lipids, because serum lipids spontaneously autooxidize to form lipid peroxides. Maseki et al.[25] demonstrated that as the serum concentrations of total lipids increase during pregnancy, so also do the concentrations of lipid peroxides, meaning that the ratio of lipid peroxides to total lipids does not change. Lipid peroxidation is also induced in the placenta during pregnancy[31,32]. Lipid peroxides originating from both the trophoblast and the villous core compartments[33] are secreted into the maternal effluent, possibly adding to levels in the maternal blood as additional peroxidation cascades are initiated.

There are conflicting reports regarding antioxidant activities changes throughout gestation. Selenium and glutathione peroxidase, both components of the antioxidant system, are decreased during pregnancy[34]. However, as in our study, the antioxidant enzyme superoxide dismutase increases in activity throughout normal pregnancy[35]. The protective antioxidant mechanisms are complex and multifactorial. The susceptibility of cells to oxidative stress is a function of the overall balance between the degree of oxidative stress and the antioxidant defense capability. It is possible that during gestation, the increase in antioxidant activity occurs in response to the normal oxidative stress due to pregnancy. At present, the nature of this mechanism is not known.

Previous studies that provided data on lipid peroxide markers during pregnancy and post partum have shown a decrease in at least one marker after delivery[6,7,10,36]. Davidge et al.[10] and Hubel et al.[6] also demonstrated a decrease in antioxidant activities after delivery compared with those in the third trimester. These results suggest that the placenta may be a source of maternal lipid peroxides. However, since all of these measurements were performed from one to three days after delivery, there is no data obtained immediately after delivery. Indeed, our results on maternal lipid peroxidation levels and antioxidant enzymatic activities at 48 hours post partum are in good agreement with these studies. However, our data on lipid peroxidation levels obtained immediately after delivery are slightly increased with a marked increase in antioxidant enzymatic activities. These findings make it likely that uncontrolled lipid peroxidation caused by

reactive oxygen species, which are produced in consequence of tissue reoxygenation, may occur during labor.

During normal respiration, the human body produces oxygen free radicals[37]. When reactive oxygen free radicals interact with the polyunsaturated fatty acids in membranes or lipoproteins, the process of lipid peroxidation begins. In the result of lipid peroxidation chain, fatty acids are converted to the primary product of lipid hydroperoxides (commonly termed lipid peroxides) and to secondary metabolites such as malondialdehyde. Under physiological condition, antioxidant defense systems have evolved to counterbalance their toxic actions by limiting the amount of lipid peroxides that can be formed[30]. On the other hand, during labor, even in normal progress, it is known that oxygenation of both maternal and fetal tissue oscillate frequently[11]. Minnich et al.[14] suggest that there are often periods of apnea and/or shallow respiration between contractions. These aberrations and hyperventilation-induced hypocarbia are probably the cause of oxygen desaturation[14]. Furthermore, it has also been confirmed that ischemia-reperfusion in human and other species leads to production of free radicals[38-41]. Uterine blood flow is directly related to the pressure difference that exists between the uterine artery and the uterine veins; that means, either a fall in uterine artery pressure[15-17] or a rise in uterine vein pressure[17] results in a proportional fall in blood flow. During uterine contractions this relationship between pressures in the uterine artery and vein and blood flow no longer holds. In addition to this, previous investigators have examined the maternal response to the pain and the stress of labor in terms of the release of certain hormones, and have reported increases in epinephrine and norepinephrine throughout labor[42,43]. Both epinephrine[44] and norepinephrine[45] cause a reduction in uterine blood flow. These evidences support our results in which a slight increase in lipid peroxide levels with a marked increase in antioxidant levels immediately after delivery were demonstrated. It also indicates that prolonged labor may cause maternal oxidative stress, because our results demonstrated that the relative changes in antioxidant enzymatic activities correlate significantly with the duration of labor.

We acknowledge that because of the relatively small number of subjects, our series is probably too limited to determine the pathogenesis of uncontrolled lipid peroxidation during labor. However, we believe that uncontrolled lipid peroxidation may result from the stress and pain of labor and suggest the need for further study.

References

1. Halliwell B: Free radicals, antioxidants, and human disease: curiosity, cause, orconsequence? Lancet 1994; 344: 721–724.
2. Halliwell B, Chirico S: Lipid peroxidation: its mechanism, measurement, and significance. Am J Clin Nutr 1993; 57 (Suppl): 715 S–725 S.
3. Wang Y, Walsh SW, Guo J, Zhang J: The imbalance between thromboxane and prostacyclin in preeclampsia is associated with an imbalance between lipid peroxides and vitamin E in maternal blood. Am J Obstet Gynecol 1991; 165: 1695–1700.
4. Wang Y, Walsh SW, Kay HH: Placental lipid peroxides and thromboxane are increased and prostacyclin is decreased in women with preeclampsia. Am J Obstet Gynecol 1992; 167: 946–949.
5. Tabacova S, Little RE, Balabaeva L, Pavlova S, Petrov I: Complications of pregnancy in relation to maternal lipid peroxides, glutathion, and exposure to metals. Reprod Toxicol 1994; 8 : 217–224.
6. Hubel CA, McLaughlin MK, Evans RW, Hauth BS, Sims CJ, Roberts JM: Fasting serum triglycerides, free fatty acids, and malonialdehyde are increased in preeclampsia, are positively correlated, and decrease within 48 hours post partum. Am J Obstet Gynecol 1996; 174: 975–982.
7. Hubel CA, Kozlov AV, Evans RW, Davidge ST, McLaughlin MK, Roberts JM: Decreased transferrin and increased transferrin saturation in sera of women with preeclampsia: Inplications for oxidative stress. Am J Obstet Gynecol 1996; 175: 692–700.
8. Walsh SW: Maternal-placental interactions of oxidative stress and antioxidants in preeclampsia. Semin Reprod Endocrinol 1998; 16: 93–104.
9. Wisdom SJ, Wilson R, Mckillop JH, Walker JJ: Antioxidant systems in normal pregnancy and in pregnancy-induced hypertension. Am J Obstet Gynecol 1991; 165: 1701–1704.
10. Davidge ST, Hubel CA, Brayden RD, Capeless EC, McLaughlin MK: Sera antioxidant activity in uncomplicated and preeclamptic pregnancies. Obstet Gynecol 1992; 79: 897–901.
11. Stipek S, Mechurova A, Crkovska J, Zima T, Platenik J: Lipid peroxidation and superoxide dismutase activity in umbilical and maternal blood. Biochem Mol Biol Int 1995; 35: 705–711.
12. Novy MJ, Edwards MJ: Respiratory problems in pregnancy. Am J Obsts Gynecol 1967; 99: 1024–1045.
13. Tomlinson MK, Caruthers TJ, Whitty JE, Gonik B: Does delivery improve maternal condition in the

respiratory-compromised gravida? Obstet Gynecol 1998; 91: 108–111.
14. Minnich ME, Brown M, Clark RB, Miller FC, Thompson DS: Oxygen desaturation in women in labor. J Reprod Med 1990; 35: 693–696.
15. Greiss FCJ: Pressure-flow relationship in the gravid uterine vascular bed. Am J Obstet Gynecol 1966; 96: 41–46.
16. Greiss FCJ, Anderson SG, King LC: Uterine pressure-flow relationships during early gestation. Am J Obstet Gynecol 1976; 126: 799–804.
17. Berman WJ, Goodlin RC, Heymann MA, Rudolph AM: Relationships between pressure and flow in the umbilical and uterine circulations of the sheep. Circ Res 1976; 38: 262–269.
18. Yagi K: Assay for serum lipid peroxide level and its clinical significance. In: Yagi K, ed. Lipid peroxides in biology and medicine. 1982. pp223–242, Academic Press, New York.
19. Wang Y, Walsh SW, Guo J, Zang J: Maternal levels of prostacyclin, thromboxane, vitamin E, and lipid peroxides throughout normal pregnancy. Am J Obstet Gynecol 1991; 165: 1690–1694.
20. Flohe L, Otting F: Superoxide dismutase assays. Methods Enzymol 1984; 105: 93–104.
21. Flohe L, Gunzler WA: Assays of glutathione peroxidase. Methods Enzymol 1984; 105: 114–120.
22. Aebi H: Catalase. Methods Enzymol 1984; 105: 121–126.
23. Marzatico F, Pansarasa O, Bertorelli L, Somenzini L, Della Valle G: Blood free radical antioxidant enzymes and lipid peroxides following long-distance and lactacidemic performances in highly trained aerobic and sprint athletes. J Sports Med Phys Fitness 1997; 37: 235–239.
24. Ishihara M: Studies on lipoperoxide of normal pregnant women and of patients with toxemia of pregnancy. Clin Chem Acta 1978; 84: 1–9.
25. Maseki M, Nishigaki I, Hagiwara M, Tomoda Y, Yagi K: Lipid peroxide levels and lipid content of serum lipoprotein fractions of pregnant subjects with or without preeclampsia. Clin Chem Acta 1981; 115: 155–161.
26. Iioka H, Akada S, Hisanaga H, Shimamoto T, Yamada Y, Moriyama I, Ichijo M: Changes in plasma levels of lipid peroxide and vitamin E during pregnancy. Asia Oceania J Obstet Gynecol 1991; 17: 357–361.
27. Loverro G, Greco P, Capuano F, Carone D, Cormio G, Sevaggi L: Lipoperoxidation and antioxidant enzymes activity in pregnancy complicated with hypertension. Eur J Obstet Gynecol Reprod Biol 1996; 70: 123–127.
28. Morris JM, Gopaul NK, Endresen MJ, Knight M, Linton EA, Dhir S, Anggard EE, Redman CWG: Circulating markers of oxidative stress are raised in normal pregnancy and pre-eclampsia. Br J Obstet Gynecol 1998; 105: 1195–1199.
29. Gladen BC, Tabacova S, Baird DD, Little RE, Balabaeva L: Variability of lipid hydroperoxides in pregnant and nonpregnant women. Reprod Toxicol 1999; 13: 41–44.
30. Little RE, Gladen BC: Levels of lipid peroxides in uncomplicated pregnancy: A review of the literature. Reprod Toxicol 1999; 13: 347–352.
31. Walsh SW, Wang Y: Secretion of lipid peroxides by the human placenta. Am J Obstet Gynecol 1993; 169: 1462–1466.
32. Poranen A-K, Ekblad U, Uotila P, Ahotupa M: The effevt of vitamin C and E on placental lipid peroxidantion and antioxidative enzymes in perfused placenta. Acta Obstet Gynecol Scand 1998; 77: 372–376.
33. Walsh SW, Wang Y: Trophoblast and placenta villous core production of lipid peroxides, thromboxane, and prostacyclin in preeclampsia. J Clin Endocrinol Metab 1995; 80: 1888–1893.
34. Behne D, Wolters SL: Selenium content and glutathione peroxidase activity in the plasma and erythrocytes of nonpregnant and pregnant women. J Clin Chem Clin Biochem 1979; 17: 133–135.
35. Sekiba K, Yoshioka T: Chages of lipid peroxidation and superoxide dismutase activity in the human placenta. Am J Obstet Gynecol 1979; 135: 368–371.
36. Wu JJ: Lipid peroxidation in preeclamptic and eclamptic pregnancies. Eur J Obstet Gynecol Reprod Biol 1996; 64: 51–54.
37. Gunter KK, Gunter TE: Transport of calcium by mitochondria. J Bioenerge Biomembrane 1994; 26: 471–485.
38. MacCord JM: Oxygen derived free radicals in post–ischemic tissue injury. N Engl J Med 1985; 312: 159–163.
39. Siesjo BK, Agardh C-D, Bengtsson F: Free radicals and brain damage. Cerebrovasc Brain Metab Review 1989; 1 : 165–211.
40. Floyd RA: Role of oxygen free radicals in carcinogenesis and brain ischemia. FASEB J 1990; 4 : 2587–2597.
41. Zini I, Tomasi A, Grimaldi R, Vannini V, Agnati LE: Detection of free radicals during brain ischemia and reperfusion by spin trapping and microdialysis. Neurosci Lett 1992; 138: 279–282.
42. Lederman PR, McCann DS, Work B: Endogenous plasma epinephrine and norepinephrine in last–trimester pregnancy and labor. Am J Obstet Gynecol 1977; 129: 5–9.
43. Falconer AD, Powles AB: Plasma noradrenaline levels during labor: Influence of elecyive lumbar epidural blockade. Anaesthesia 1982; 37: 416–421.
44. Rosenfeld CR, Barton MD, Meschia G: Effects of epinephrine on distribution of blood flow in the pregnant ewe. Am J Obstet Gynecol 1976; 124: 156–160.
45. Rosenfeld CR, West J: Circulatory response to systemic infusion of norepinephrine in the pregnant ewe. Am J Obstet Gynecol 1977; 127: 376–381.

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