Original Article

Thioredoxin-1 and oxidative stress status in pregnant women at early third trimester of pregnancy: relation to maternal and neonatal characteristics

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This study examined the clinical and biological importance of thioredoxin-1, a redox-active defensive protein that controls multiple biological functions, in pregnant women. We measured serum concentrations of thioredoxin-1, total hydroperoxides, and redox potential in 60 pregnant women at the early third trimester: gestational age of 27–29 weeks. The thioredoxin-1 concentration (mean ± SD) was 90 ± 42 ng/ml. Total hydroperoxides was 471 ± 105 U.CARR (1 U.CARR = 0.08 mg/dl H_2O_2). Redox potential was 2142 ± 273 µmol/l. The total hydroperoxides: redox potential ratio (oxidative stress index) was 0.23 ± 0.08. Thioredoxin-1, total hydroperoxides, and oxidative stress index were higher and redox potential was lower than in blood of healthy adults. Total hydroperoxides and redox potential were mutually correlated significantly and negatively. Thioredoxin-1 correlated significantly and negatively and redox potential correlated significantly and positively with body weight and body mass index. Thioredoxin-1 and redox potential correlated significantly and positively with uric acid and albumin, respectively. Thioredoxin-1 and oxidative stress index correlated significantly and negatively and redox potential significantly and positively with neonatal birth weight. These results suggest that high concentrations of thioredoxin-1 are linked to high oxidative stress status in pregnant women and that neonatal birth weight is affected by the maternal oxidative condition during later pregnancy.

Key Words: later pregnancy, oxidative stress, redox potential, thioredoxin, total hydroperoxides

Aerobic organisms normally generate reactive oxygen-derived species (ROS) during respiration and metabolism processes. However, when the generation of ROS exceeds the antioxidant defense capacity, ROS can damage cellular macromolecules including proteins, lipids, and nucleic acids.[1] Increased generation of ROS during growth of the fetal-placental unit is a prominent feature of pregnancy.[2–6] Further enhancement of oxidative stress is likely to promote several pregnancy-related disorders including preeclampsia, fetal growth restriction (FGR), preterm labor, and low birth weight.[7,8]

Thioredoxin (TRX) is a ubiquitously expressed, multifunctional protein (12-kDa) that has a redox-active dithiol-disulfide within the conserved -Cys-Gly-Pro-Cys- sequence.[9] This defensive protein is induced in response to various stress conditions.[9,10] Thioredoxin plays a crucial role in ROS detoxification and transcription factor regulation, each of which is critical to normal cellular function. Human-TRX-overexpressing transgenic mice survive longer[11] and are more resistant to various oxidative conditions than control mice are.[12–14]

Thioredoxin is expressed in human decidua and trophoblasts, where it might protect the fertilized egg and placental trophoblasts from the cytotoxic effects of ROS.[15,16] Histological examination of the human placenta has revealed that TRX is expressed in cytotrophoblasts, decidual cells, and stromal cells in the stem villi.[17] Moreover, examination of human fetal tissues has revealed that TRX is distributed widely in different tissues and organs.[18] It is noteworthy that complete deficiency of cytosolic TRX, TRX-1, results in early embryonic lethality in mice.[19] Overexpression of TRX-1 reduces oxidative stress in the placenta of transgenic mice and TRX-1 might promote normal growth and development in the mouse fetus.[20,21]

These findings suggest that TRX plays a role in growth and development of the fetal-placental unit in humans. For this study, we measured serum concentrations of TRX-1 in pregnant women at the early third trimester of pregnancy. We determined total hydroperoxides (TH) and redox potential (RP)[22] in the sera for comparison. We also examined whether the concentrations of those parameters were associated with subsequent pregnancy and birth outcomes. This report describes high serum concentrations of TRX-1 and TH in pregnant women and presents findings related to maternal and neonatal characteristics.

Materials and Methods

Participants. During August 2011–November 2011, pregnant women at the early third trimester were asked to participate in this study. They had visited Perineito Hahatokono Hospital for Mothers and Children, which is located in Okayama, Japan. Exclusion criteria were the following: non-Japanese, date of last menstrual period uncertain, age <20 years or ≥40 years, multiple pregnancy, smoking or drinking habits, intake of multivitamins, hypertensive (systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg) or infectious condition, history of chronic condition (such as cardiovascular, renal, metabolic, immunological, and neurological diseases).

In this study, 60 pregnant women were enrolled. Each gave written informed consent before entering the study. The study, which was approved by the local ethics committee, was performed according to the Declaration of Helsinki.

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Clinical and laboratory data. Gestational age (GA) was estimated based on reported data of the subject's last menstruation and ultrasound measurements performed by obstetricians. Body weight and height, by which the body mass index (BMI) was calculated, and blood pressure were recorded by trained nurses. Non-hemolytic venous blood was sampled for measurement of AST, ALT, LD, creatinine, uric acid, albumin, and glucose using automated analyzers with commercially available kits. Thioredoxin-1 and other oxidative stress biomarkers were measured in the sera using the methods described below.

Assay of TRX-1. Thioredoxin-1 was measured using a sensitive sandwich ELISA system (Redox Bioscience Inc., Kyoto, Japan) according to the procedure described previously.[10,23] Two specific mouse monoclonal antibodies to non-overlapping epitopes of human TRX-1 (ADF 11 and ADF 21) were used. These antibodies do not cross-react to mitochondrial TRX, TRX-2. Nakamura and coworkers[10] reported that serum concentrations of TRX-1 are 10–30 ng/ml in healthy adults, rising to 40–140 ng/ml in patients presenting with diseases characterized by enhancement of oxidative stress.[10] In our laboratory, serum TRX-1 concentrations in 13 healthy Japanese adults (female/male: 7/6) aged 32 ± 7 years (18–43 years) determined using this methodology were 20 ± 17 ng/ml (5–53 ng/ml).

Assay of TH and RP. Total hydroperoxides and RP were measured using the Free Radical Analytical System (Diacron International, Grosseto, Italy) according to procedures detailed previously.[22,24] Total hydroperoxides represent the peroxidation metabolites of proteins, peptides, amino acids, lipids, and fatty acids. The TH values were measured using the d-ROM (Reactive Oxygen Metabolites) kit that applies Fenton and Haber–Weiss reactions, where one U.CARR (unit of TH value) is equivalent to 0.08 mg/dl of H_2O_2. Reference values reported by the manufacturer are 250–300 U.CARR. In healthy Japanese adults, serum TH values are 275 ± 48 U.CARR for those aged 20–29 years ($n = 80$) and 283 ± 50 U.CARR ($n = 118$) for those aged 30–39 years.[24]

Redox potential indicates the total endogenous (such as albumin, transferrin, bilirubin, uric acid) and exogenous (such as ascorbate, carotenes, tocopherols, ubiquinol) antioxidant capacity. The RP values were measured using the BAP (Biological Antioxidant Potential) kit, which determines the concentration of antioxidants as agents that reduce iron ion from its ferric (Fe^{3+}) to ferrous (Fe^{2+}) form. The intensity was estimated photometrically and the result was expressed as μmol/l. Reference values reported by the manufacturer are more than 2200 μmol/l. In healthy Japanese adults, serum RP values are 2546 ± 59 μmol/l for those aged 20–29 years ($n = 80$) and 2554 ± 57 μmol/l ($n = 118$) for those aged 30–39 years.[24]

The ratio of TH to RP was calculated and designated as "oxidative stress index (OSI)" because the shift of the oxidant/antioxidant balance toward the oxidative side represents oxidative stress.[25] The rough estimation of OSI was around 0.1 in healthy Japanese adults, as calculated from data reported by Nojima and coworkers.[24]

Neonatal characteristics at birth. The recorded neonatal characteristics were the following: mode of delivery, sex, GA, birth weight (BW) and BW SD score at each GA,[26] Apgar scores, pH and HCO_3^- value in umbilical cord blood.

Statistical analysis. Data are presented as mean $\pm$ SD and/or range (minimum–maximum). Differences between groups were examined using unpaired t test. Correlations between variables were assessed using Pearson correlation coefficients. Differences for which $p<0.05$ were regarded as significant. All statistical calculations were performed using GraphPad Prism 5.0 (GraphPad Software Inc., San Diego, CA).

Results

Characteristics of the 60 subjects. The age of the subjects

Table 1. Characteristics of 60 pregnant women at the early third trimester

Age (years)	31 ± 5 (21–39)
Gestational age (weeks)	28 ± 1 (27–29)
Weight (kg)	59 ± 9 (46–95)
Height (m)	159 ± 6 (145–173)
Body mass index (kg/m²)	23 ± 3 (18–35)
Systolic blood pressure (mm Hg)	96 ± 9 (72–123)
Diastolic blood pressure (mm Hg)	54 ± 8 (23–71)
AST (IU/l)	15 ± 3 (10–28)
ALT (IU/l)	10 ± 5 (4–27)
LD (IU/l)	167 ± 18 (132–217)
Creatinine (mg/dl)	0.45 ± 0.06 (0.35–0.58)
Uric acid (mg/dl)	3.1 ± 0.6 (1.8–4.9)
Albumin (g/dl)	3.4 ± 0.2 (3.0–3.9)
Glucose (mg/dl)	77 ± 11 (64–137)

Data are expressed as mean ± SD and range.

Table 2. Serum concentrations of thioredoxin-1 and other oxidative stress biomarkers in 60 pregnant women

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Thioredoxin-1 (ng/ml)	90 ± 42 (11–205)
Total hydroperoxides (U.CARR)	471 ± 105 (193–708)*
Redox potential (μmol/l)	2142 ± 273 (1430–2601)*
Oxidative stress index	0.23 ± 0.08 (0.09–0.45)

Data are expressed as mean ± SD and range. "Oxidative stress index" is defined as the ratio of total hydroperoxides (U.CARR) to redox potential (μmol/l). *A significant and negative correlation was found between total hydroperoxides and redox potential. ($r = -0.46$, $p = 0.0002$).

was 31 ± 5 years (21–39 years). Of the 60 women, 25 women (42%) were primigravid and 35 (58%) were multigravid. Venous blood was sampled at the GA of 28 ± 1 weeks (27–29 weeks). They showed no abnormal results for serum AST, ALT, LD, creatinine, uric acid, albumin, or glucose. Clinical and laboratory data of the subjects are presented in Table 1.

Serum concentrations of TRX-1, TH, RP, and OSI. Table 2 shows the serum concentrations of TRX-1, TH, RP, and OSI in the pregnant women at the early third trimester. The TRX-1 was 90 ± 42 ng/ml (11–205 ng/ml). TH was 471 ± 105 U.CARR (193–708 U.CARR). RP was 2142 ± 273 μmol/l (1430–2601 μmol/l). OSI was 0.23 ± 0.08 (0.09–0.45). Correlations between the parameters were the following: $r = -0.10$, $p = 0.43$ for TRX-1 vs TH; $r = -0.03$, $p = 0.85$ for TRX-1 vs RP; $r = -0.07$, $p = 0.57$ for TRX-1 vs OSI; $r = -0.46$, $p = 0.0002$ for TH vs RP (Fig. 1). A significant and negative correlation was found between TH and RP in the subjects.

At the time of blood sampling, 18 pregnant women were taking oral ritodrine. One woman was taking low-dosage aspirin. No significant difference was found in the levels of TRX-1, TH, RP, and OSI between the women taking medication ($n = 19$) and those taking no medication ($n = 41$) (data not shown).

Correlations between oxidative stress biomarkers and clinical and laboratory data. Correlations between oxidative stress biomarkers (TRX-1, TH, RP, OSI) and clinical data (age, body weight, height, BMI, blood pressures) were assessed. Statistical significance was found only for TRX-1 vs BW ($r = -0.27$, $p = 0.036$), TRX-1 vs BMI ($r = -0.26$, $p = 0.041$), RP vs BW ($r = 0.33$, $p = 0.010$), and RP vs BMI ($r = 0.36$, $p = 0.005$). The respective correlations between oxidative stress biomarkers and biochemistry values (AST, ALT, LD, creatinine, uric acid, albumin, glucose) were also assessed. Statistical significance was found only for TRX-1 vs uric acid ($r = 0.34$, $p = 0.008$) and RP vs albumin ($r = 0.29$, $p = 0.022$).
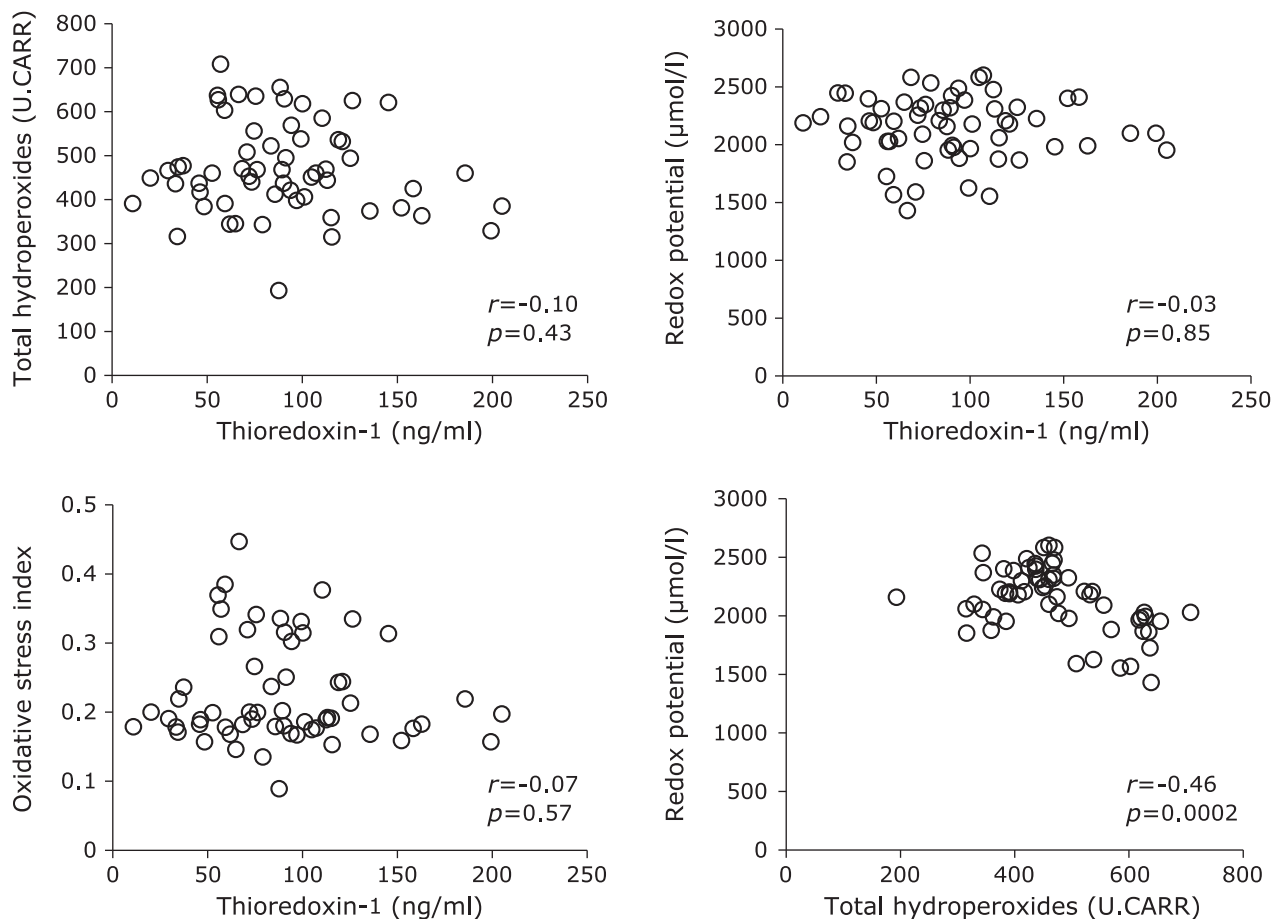
Fig. 1. Correlations between thioredoxn-1 and other oxidative stress biomarkers in 60 pregnant women. A significant and negative correlation was found between thioredoxin-1 and redox potential.

Relation with pregnancy outcome. Of the 60 women, three subsequently developed nonsevere hypertension. No significant difference was identifiable in those levels between the women who developed hypertension ($n = 3$) and those who did not ($n = 57$). The TRX-1 was 79 ± 44 ng/ml and 90 ± 42 ng/ml, TH was 425 ± 97 U.CARR and 473 ± 105 U.CARR, RP was 2161 ± 251 μmol/l and 2141 ± 276 μmol/l, and OSI was 0.20 ± 0.05 and 0.23 ± 0.08, respectively. None of the 60 women developed diabetes mellitus or other critical disorder during later pregnancy.

Relation with birth outcome. None of the 60 women gave birth to infants with congenital anomalies or those requiring intensive clinical care such as neonatal asphyxia, respiratory distress syndrome, sepsis, or organ failure. The neonatal characteristics are presented in Table 3. Of the neonates, 56 were born at term and 4 were born preterm. No neonate had been asphyxiated at birth. Their Apgar scores and pH and HCO₃⁻ values in umbilical cord blood were not abnormally low. When correlations between oxidative stress biomarkers and the BW or BW SD score of the neonates were tested, statistical significance was found for TH vs BW ($r = -0.26$, $p = 0.046$), RP vs BW ($r = 0.31$, $p = 0.018$), and OSI vs BW ($r = -0.33$, $p = 0.010$). Fetal growth restriction criteria are BW less than the tenth percentile of standard values at each GA.[26] No significant difference was found in those levels between the FGR group ($n = 5$) and the non-FGR group ($n = 55$). The TRX-1 was 103 ± 37 ng/ml and 88 ± 43 ng/ml, TH was 477 ± 121 U.CARR and 470 ± 104 U.CARR, RP was 2125 ± 219 μmol/l and 2142 ± 279 μmol/l, and OSI was 0.23 ± 0.07 and 0.23 ± 0.08.

Table 3. Neonatal characteristics at birth

Mode of delivery	vaginal/cesarean = 43/17
Sex	female/male = 21/39
Gestational age (weeks)	39 ± 1 (35–41)
	term/preterm = 56/4
Weight (g)	3011 ± 381 (2214–4198)
Weight (SD score)	0.02 ± 0.91 (–2.35–2.38)
Apgar score (1 min)	9.0 ± 0.2 (8–10)
Apgar score (5 min)	9.9 ± 0.3 (9–10)
pH in umbilical cord blood	7.34 ± 0.05 (7.20–7.49)
HCO₃⁻ in umbilical cord blood	24 ± 2 (20–29)

Data are expressed as mean ± SD and range.

Discussion

Pregnancy *per se* is a state of oxidative stress arising from increased placental metabolic activity and increased production of ROS and relatively reduced total antioxidant capacity.[2] Concentrations of oxidative stress biomarkers, including blood lipid peroxides, oxidized LDL, and 8-isoprostane and urinary 8-hydroxydeoxyguanosine[1] became higher toward the third trimester of pregnancy than in non-pregnant women.[3,4,6] Notably, serum TH values (using the d-ROM kit) in pregnant women at 27–31 weeks of gestation were elevated (531 ± 143 U.CARR; range 153–895 U.CARR).[5] Those values showed significant and positive correlations with body weight and both systolic and

diastolic blood pressures. Regarding antioxidants, erythrocyte activities of glutathione peroxidase and superoxide dismutase increased toward the third trimester of pregnancy.[6] In contrast, the RP values determined by the ferric reducing ability of plasma test, which exploits the same chemical principle of the BAP kit, were lower in pregnant women than in non-pregnant women.[6] In addition, the serum RP values determined using 2,2'-azino-di-(3-ethylbenzthiazoline sulfonate) were reported to decrease gradually as pregnancy advances.[4] Collectively, these results indicate that the total antioxidant capacity in plasma or serum is not enhanced in spite of the presence of excessive ROS during later pregnancy.

Only one study has measured serum TRX-1 concentrations in pregnant women. Kuroda and coworkers[27] reported that serum TRX-1 concentrations are elevated during pregnancy. The TRX-1 concentrations were, respectively, 57 ± 26 ng/ml, 66 ± 25 ng/ml, and 66 ± 23 ng/ml in the first, second, and third trimesters of pregnancy, as compared to the non-pregnant control values (48 ± 26 ng/ml). The physiological increase of TRX-1 concentrations supports the contention that TRX-1 is involved in the maintenance of normal pregnancy in humans.[15–18] Our earlier report described that the TRX-1 concentrations in umbilical cord blood (127 ± 81 ng/ml) and those in early breast milk (268 ± 149 ng/ml) are elevated remarkably.[23] These results indicate that the systemic release of TRX-1 is enhanced in neonates at birth and that early breast milk is a rich source of this protein.

In this study, we measured serum TRX-1 concentrations in 60 pregnant women at the early third trimester. Results showed that their TRX-1 concentrations (90 ± 42 ng/ml) were several times higher than healthy adult values (10–30 ng/ml).[10] The results are consistent with data presented by Kuroda and coworkers.[27] We measured the oxidative stress status further in pregnant women using d-ROM and BAP tests. The TH values were higher, but the RP values were lower in the pregnant women, as compared to healthy adult values.[24] Accordingly, the OSI (0.23 ± 0.08) was about two times higher than the healthy adult level (around 0.1)[25] The RP values were found to have significant and negative correlation with the TH values in the subjects, thereby implying that antioxidant buffering capacity is attenuated as the oxidative load increases. The results suggest that the high concentrations of TRX-1 are linked to physiologically high oxidative stress status and reduced antioxidant capacity in pregnant women, although the TRX-1 concentrations *per se* were not correlated directly with the other oxidative stress markers.

Among the correlations analyzed statistically, TRX-1 and RP respectively showed significant negative and positive correlations with each of BW and BMI in these pregnant women. Although the causal relation remains unclear, these results suggest that systemic release of TRX-1 and total antioxidant capacity in serum are related closely to maternal body size. Additionally, it is intriguing that TRX-1 and RP showed significant and positive correlations with uric acid and albumin, respectively, in the subjects. Uric acid is recognized as a marker of oxidative stress. Uric acid functions not only as an antioxidant but also as a pro-oxidant.[28] The physiological role of uric acid in the fetal-placental unit might be complex and multifactorial. The BAP kit does not measure individual antioxidants. It measures the overall effect of many non-enzymatic antioxidants.[22] Albumin, which is present in high concentrations in blood, scavenges various strong oxidants. Our results support the contention that albumin contributes substantially to the RP value determined by the BAP kit.[29]

Enhancement of oxidative stress during pregnancy has emerged as a likely promoter of preeclampsia, diabetes mellitus, FGR, and other pregnancy-related disorders.[30–32] Previous histological examinations revealed that the levels of 4-hydroxynonenal, TRX, glutaredoxin, and protein disulfide isomerase were increased in the placenta in preeclampsia than in uncomplicated pregnancy[33] and that levels of 8-hydroxydeoxyguanosine and TRX were higher in the placenta in cases of preeclampsia or FGR than in uncomplicated pregnancy during the third trimester.[34] These results indicate that TRX might be induced adaptively against oxidative stress in the placenta in preeclampsia and FGR.

In this study, no significant difference was found in the biomarkers of women who developed hypertension and those who did not, or between those who developed FGR and those who did not. We must admit that the subjects with critical complications were very few. The predictive value of serum TRX-1 (together with TH, RP, OSI) for the development of pregnancy-related disorders should be evaluated thoroughly in future studies.

Thioredoxin is known to be expressed widely in the human female reproductive system, including placenta,[15–17] fetus,[18] ovary,[35] endometrium,[36] and cervix.[37] The increase in TRX expression occurs preferentially in the functionally active tissues and cells in this system. Reportedly, TRX plays a role in reproduction as a component of the "early pregnancy factor".[38] Target disruption of the mouse TRX-1 gene results in early embryonic lethality.[19] Moreover, TRX-1 is likely to be synthesized in high amounts in breast tissues of lactating women.[23] These findings, together with our present results, indicate that TRX-1 provides a unique protective mechanism that allows the maintenance of redox balance in the female reproductive processes and outcomes.

This study has some limitations that must be addressed in future research. First, our study involves a small population, with no direct measurement of fetal or neonatal oxidative stress status. Second, information about dietary habits of the subjects was not available. Third, the samples are not representative of pregnant women in general because the data were of a hospital-based study. Finally, the study design is limited by its cross-sectional nature. A longitudinal study is necessary to ascertain the predictive ability of the parameters for pregnancy and birth outcomes.

In conclusion, this study documents that serum concentrations of TRX-1, TH, and OSI are higher and RP is lower in pregnant women at the early third trimester than in healthy adults and that the TH and RP correlate significantly and negatively. This study also demonstrates that TRX-1 correlates significantly and negatively and RP correlates significantly and positively with body weight and body mass index in the pregnant women. Moreover, TRX-1 and OSI correlate significantly and negatively and RP correlates significantly and positively with neonatal birth weight. These results suggest that the high concentrations of TRX-1 are linked to high oxidative stress status in pregnant women and that neonatal birth weight is affected by the maternal oxidative condition during later pregnancy.

Acknowledgments

Abbreviations

BMI	body mass index
BW	birth weight
FGR	fetal growth restriction
GA	gestational age
OSI	oxidative stress index
ROS	reactive oxygen-derived species
RP	redox potential
TH	total hydroperoxides
TRX	thioredoxin

Conflict of Interest

No potential conflicts of interest were disclosed.

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