Eur J Pediatr (2011) 170:645–651
DOI 10.1007/s00431-010-1338-9

ORIGINAL PAPER

Maternal active or passive smoking causes oxidative stress in placental tissue

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Received: 13 July 2010 / Accepted: 12 October 2010 / Published online: 28 October 2010

Abstract The aim of this study was to assess the influence of active and passive maternal smoking on placenta total oxidant/antioxidant status in term infants. The levels of cord blood total antioxidant capacity (TAC), total oxidant status (TOS), and oxidative stress index (OSI) were measured in samples of fetal placental tissue, cord blood, and the maternal peripheral blood serum and from 19 mothers who were active smokers, 19 who were passive smokers, and 22 who were nonsmokers (not exposed to active or passive smoking). The pregnancies were between 37 and 40 weeks' gestation, were uncomplicated, and the infants were delivered vaginally. Birth weight and head circumference in the active smokers were significantly ($P<0.001$) lower than those in the controls. Placenta, cord blood, and the maternal peripheral TAC levels were significantly lower in the active smokers compared with the controls ($P<0.001$), while TOS and OSI levels were significantly higher in the active and passive smokers than in the controls ($P<0.001$). A positive significant correlation was found between active maternal smoking and placenta TOS and OSI levels ($P<0.016$), and a significant negative correlation was found between number of cigarettes exposed to and birthweight and head circumference ($P<0.05$). In conclusion, active or passive maternal smoking is associated with important alterations in oxidant and antioxidant balance in fetal placental tissue and causes potent oxidative stress.

Keywords Antioxidants · Cord blood · Oxidants · Oxidative stress · Passive smoking · Placenta · Smoking

Abbreviations

TAC	total antioxidant capacity
TOS	total oxidant status
OSI	Oxidative stress index

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Introduction

Active or passive smoke contains numerous compounds emitted in gases, many of which are oxidants and pro-oxidants, and is associated with numerous adverse pregnancy outcomes, including intrauterine growth retardation and fetal death [23, 27]. The enhanced production of reactive oxygen species by smoke is related to increased free radical production, depleted serum antioxidants, and oxidative stress [3, 6, 18, 28], which can result in the oxidation of lipids, induction of DNA single-strand breakage, inactivation of certain proteins, and the disruption of biological membranes [14, 26, 36]. Active or passive smoking causes oxidative stress in pregnant women and may have a similar effect in fetuses [6, 27]. We previously

reported that total antioxidant capacity (TAC) and total oxidant status (TOS) and oxidative stress index (OSI) in infants and their mother's serum and fetus cord blood are altered by active or passive smoking [3, 4, 27]. In the present study, we evaluated further the effect of maternal active and passive smoking throughout pregnancy on the levels of antioxidant and oxidant status in placental tissue collected at uncomplicated term birth.

Subjects and methods

Subjects

Placental tissue, cord blood, and maternal peripheral venous blood samples were obtained from 60 women at 37–40 weeks' gestation at delivery. The study included a consecutive series of 19 active smokers, who smoked 4±2 cigarettes per day, a series of 19 passive smokers, who passive smoked 17±4 cigarettes per day, and a series of 22 nonsmokers, who had never been exposed to active or passive smoking.

The women's mean age was 26±3 years, they had no medical history, and attended Sanliurfa Women's Hospital. A brief history was taken, and a complete physical examination was performed. Newborns with an Apgar score <7 at 10 min, documented infection or respiratory distress, or any signs or symptoms of any acute or chronic illness were excluded from the study along with their mothers. All subjects were of similar socioeconomic status and were living in a rural region. None had taken any antioxidant medications (vitamin C, vitamin E, selenium, etc.) or had drunk fruit juice prior to or during the study. Gestational age was determined from the date of the last menstrual period and confirmed by the infant's maturation findings.

The Harran University Medical Faculty ethics committee approved the collection of placental tissue, umbilical cord blood, and maternal peripheral venous blood for research. The department is conducting ongoing research projects on cord blood. The mothers were fully informed about the aim of the investigation and, after their consent to be involved in the study was obtained, approximately 1 g of placental tissue, and 3–4 ml each of cord blood and maternal peripheral blood was aspirated from the vein into syringes, and serum was separated from the cells by centrifugation at 1,500×*g* for 10 min. All samples were stored at –80°C without preservative until assayed.

Tissue sampling and homogenization

Before biochemical assays, placental tissues were weighed, broken down into very small pieces, and placed in empty glass tubes. Then 10 ml of 140 mM KCl solution per gram of tissue was added to each tube, and then all tissues were homogenized in a motor-driven homogenizer. The homogenate was centrifuged at 2,800×*g* for 10 min at 4°C [29]. The resulting supernatant was used for the levels of TAC and TOS.

Analytical methods

Total oxidant status (TOS) and total antioxidant capacity (TAC) levels were measured by Erel's methods, which are automated and colorimetric [9, 15, 17]. Erel's TOS method is based on the oxidation of ferrous ion to ferric ion in the presence of various oxidant species in acidic medium and the measurement of the ferric ion by xylenol orange. Erel's TAC method is based on the bleaching of the characteristic color of a more stable 2,2′-azino-bis (3-ethylbenz-thiazoline-6-ulfonic acid) (ABTS) radical cation by antioxidants [1]. The results of peripheral blood and cord blood serum TOS and TAC were expressed in micromoles H_2O_2/l and millimoles Trolox Equiv. per liter, respectively, and the precision of this assay is excellent—lower than 3% [16, 22]. The percentage of TOS level to TAC level was regarded as the oxidative stress index (OSI) [5, 19]. The unit of placental tissue TOS and TAC was μmole H_2O_2 Equiv./gram protein and mmole H_2O_2 Equiv./gram protein, respectively [26, 29]. The placental tissue OSI value was calculated as follows: OSI=[(TOS, μmole H_2O_2 Equiv./gram protein)/(TAC, μmole H_2O_2 Equiv./gram protein)×100]. The serum OSI value was calculated as follows: OSI=[(TOS, μmol/l)/(TAC, μmol Trolox equivalent/l)×100] [2, 4].

Statistical analyses

Differences in the placenta and serum parameters between the active smoker, passive smoker, and control groups were analyzed by one-way ANOVA with Tukey's honestly significant difference multiple comparison test when the Levene test of homogeneity of variance statistic's significance was higher than 0.05. Sex ratio was compared using a chi-square test. Bivariate associations between variables were assessed by Pearson's correlation test. The data were expressed as mean ± SD and differences were considered statistically significant at $p<0.05$.

Statistical analyses were performed using SPSS for Windows Release 11.5 (SPSS)

Results

We found no significant differences between the groups with regard to male/female distribution, mean gestational

age, or mother's age ($P>0.05$). Body weights and length were lower in the active smoker group compared to the control group and passive smoker group (Table 1).

Placenta tissue, cord blood, and maternal peripheral blood serum TAC, TOS, and OSI levels are shown in Table 2. Placental tissue, cord blood, and maternal peripheral blood serum TOS levels were the highest in the active smoker group, lowest in the control group, and in the middle in the passive smoker group (Fig. 1). Placenta TOS levels were 396±121, 274±87, and 358±103 μmol H_2O_2 Equiv./gram protein, respectively, differing significantly between the groups ($P=0.001$).

Placenta, cord blood, and maternal peripheral blood TAC levels were significantly lower in the active smoker group than in the control group (Fig. 2) ($P<0.001$), and placental tissue TAC levels were significantly lower in the passive smoker group than in the control group ($P=0.017$), but cord blood and maternal blood TAC levels were not significantly lower in the passive smoker group than in the control group. Placenta TAC levels were 2.25±0.47, 2.64±0.58, and 3.36±0.76 mmole H_2O_2 Equiv./gram protein, respectively, and differed significantly between the active smoker group, passive smoker group, and control group ($P=0.001$). Active smokers' TAC levels were lower than those of the passive smokers but not significantly so ($P=0.143$).

Placental tissue, cord blood, and maternal blood OSI levels were highest in the active smoker group, lowest in the control group, and in the middle in the passive smoker group. Placenta TOS levels were 18.3±7.9, 9.1±3.7, and 13.9±4.4, respectively, differing significantly between the groups (Fig. 3) ($P=0.001$).

Positive significant correlations were found between maternal cigarette exposure and placenta, cord blood, and maternal peripheral blood TOS (Fig. 4) and OSI levels ($P<0.016$), while a negative significant correlation was found between number of cigarettes exposed to and birth weight and head circumference and between placenta and cord blood TAC levels ($P<0.010$)

Discussion

In the present study, we found that the oxidative/antioxidative balance shifted towards the oxidative side, namely oxidative stress was present in the study group compared to the control group. To the best of our knowledge, all of the published studies related to the oxidative effects of active or passive smoking are about children [18], infants and their mothers [3, 4], and newborn cord blood [6]; this is the first report showing an association between increased fetal placenta tissue oxidative status in mothers who were active and passive smokers.

Active smoking mothers smoked on average four cigarettes versus passive smokers, who were exposed to on average 17 cigarettes per day: yet the antioxidant and oxidant status seemed to be more pronounced in the active smokers. The quantities of the smoke-derived chemicals such as oxides of nitrogen, nicotine, carbon monoxide, and various carcinogens in the environment depend on a host of factors such as the number of smokers, the extent of their cigarette consumption, the rate of smoking, the type of cigarette (filter or non-filter, low tar, nicotine content, etc.), the proximity of the non-smoker, the duration of exposure, the magnitude of the space, the home or work ventilation system, the season of the year, and many other variables [7]. All these factors, combined with the results of our studies, led us to conclude that further studies are needed to more clearly identify the effects of active and passive smoking on the oxidant/antioxidant system.

Previous studies have reported the negative effect of maternal smoking on birth weight [11, 31, 35]. There is a significant dose–response relationship, and maternal exposure, even if only passive, can produce infants at risk for

Table 1 Anthropometric data of newborns, maternal age, and number of cigarettes smoked according to smoke exposure

	Baby of active smoker mother, $n=19$	Baby of passive smoker mother, $n=19$	Control, $n=22$	P^a
Maternal age	26.5±6	24.7±5	29.1±7	>0.05
Gestational age (week)	38±0.2	38±0.5	38.2±0.2	>0.05
Girl/boy	10/9	10/9	10/9	>0.05
Birth weight (g)	3.210±280[b]	3.600±220	3.460±250	<0.05
Lenght (cm)	49.5±1.2	49.1±1.2	50.3±1.42	>0.05
Head circumference (cm)	37.2±0.9[b]	37.3±0.9	38.±0.98	<0.05
Cigarettes perday	4±2	17±4	–	<0.05

Data given as mean ± SD

[a] One-way ANOVA with the Tukey's honestly significant difference multiple comparison test

[b] The difference is between active smoker and controls

Table 2 Comparison of placental tissue, cord blood, and mother's peripheral blood oxidative and antioxidative parameters in the groups

		Active smoker, n=19	Passive smoker, n=19	Control, n=22	P[a]	Posthoc[b]
TOS (μmol H2O2 Eqv./L)	Placenta tissue[c]	397±121	358±103	274±87	0.001	All
	Cord blood	22.3±5.4	15.9±3.9	12.8±1.8	<0.001	All
	Maternal blood	23.2±4.2	18.7±6	14.7±3.6	<0.001	All
TAC (μmol H2O2 Eqv./L)	Placenta tissue[d]	2.25±0.47	2.64±0.58	3.36±0.76	<0.001	AS&C, PS&C
	Cord blood	1.0±0.13	1.14±0.21	1.25±0.19	<0.001	AS&C
	Maternal blood	0.87±0.16	1.0±0.16	1.12±0.17	<0.001	AS&C
OSI (AU)	Placenta tissue	18.32±7.89	13.89±4.41	9.10±3.74	<0.001	All
	Cord blood	2.26±0.65	1.44±0.48	1.04±0.26	<0.001	All
	Maternal blood	2.75±0.74	1.88±0.62	1.34±0.40	<0.001	All

Data given as mean ± SD

TAC total antioxidant capacity, *TOS* total oxidant status, *OSI* oxidative stress index, *AS* active smoker, *PS* passive smoker, *C* control

[a] One-way ANOVA with the Tukey's honestly significant difference multiple comparison test

[b] Differences are significant between mentioned groups (P<0.05)

[c] μmole H2O2 Equiv./gram protein

[d] mmole H2O2 Equiv./gram protein

perinatal complications associated with growth restriction [31]. Varvarigou et al. described cases involving "minimal" consumption of one to five cigarettes per day [35].

They found an essential adverse effect of tobacco smoke exposure on fetal growth in pregnant women exposed to passive smoking, as well as in those with "minimal" maternal cigarette consumption. A decrease in birth weight and an increase in the prevalence of growth restriction with increasing cigarette consumption were observed. In our previous study, similar to this study, birth weight did not differ between passive smokers and controls [6]. The differences may be due to socioeconomic factors [11], maternal age [13], and BMI [34]. Maternal BMI is an important factor; an increased BMI is associated with larger birth weight. Maternal self-report is less predictive of birth weight abnormalities than other objective measures of exposure [31]. Although maternal report may be less accurate than measured levels of cotinine, particularly in

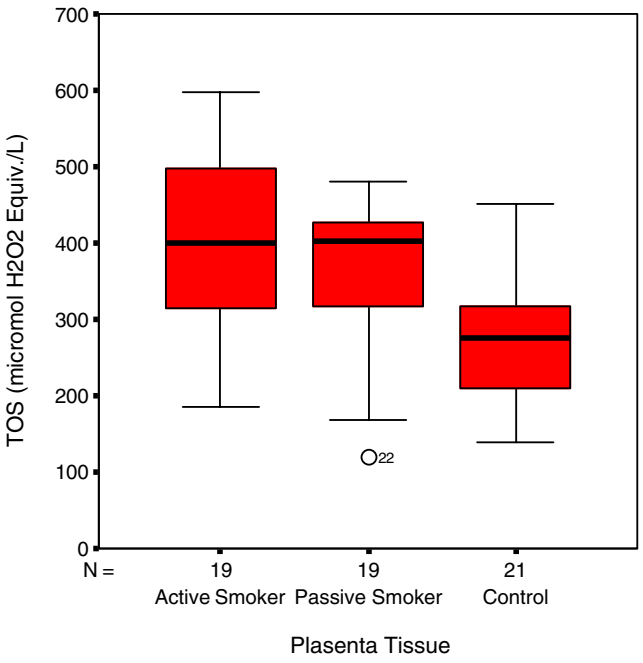

Fig. 1 Boxplot graphic of placenta tissue TOS levels in active smokers, passive smokers, and controls. Differences are significant between the groups (P<0.05)

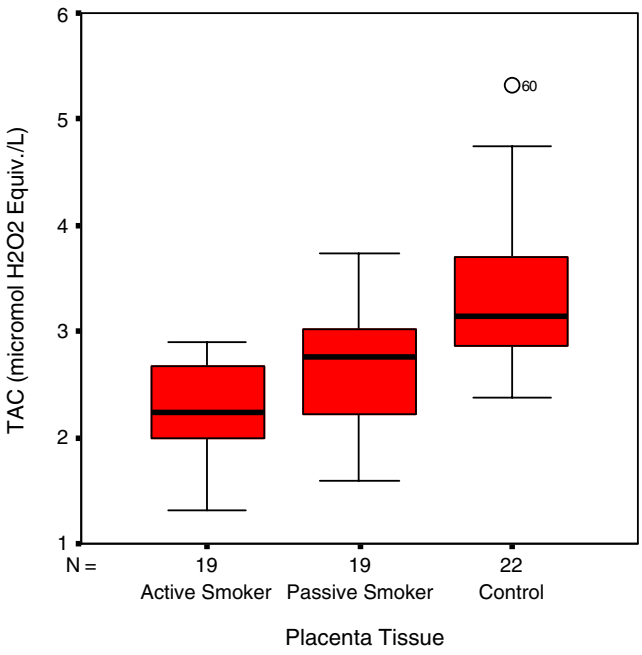

Fig. 2 Boxplot graphic of placenta TAC levels in active smokers, passive smokers, and controls. Differences are more significant between active and passive smokers than controls (P<0.05)

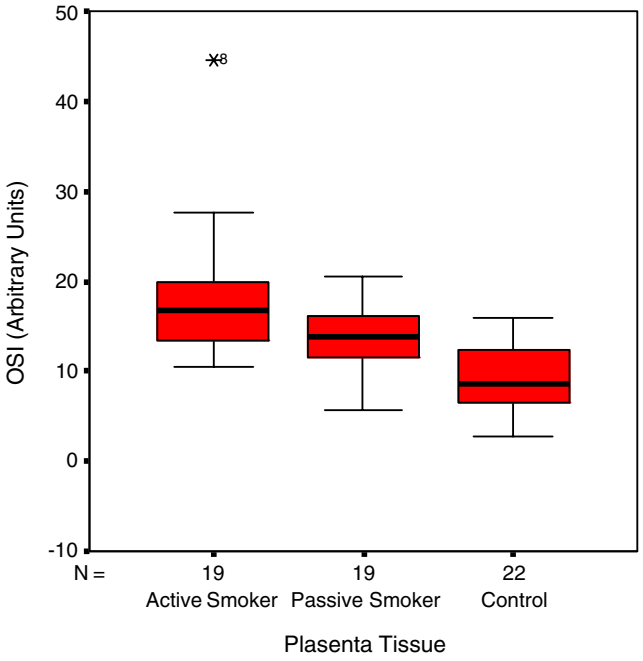

Fig. 3 Boxplot graphic of placenta tissue OSI levels in active smokers, passive smokers, and controls. Differences are significant between the groups ($P<0.05$)

patients who report quitting during pregnancy, there is a correlation between reported amount of smoking and measurable levels of cotinine.

Lipid peroxidation products are measured as an index of oxygen free radical generation. Measurement of TOS provides a sensitive index of lipid peroxidation and oxidative stress [15, 20]. It has been argued that the increased production of reactive oxygen species associated with smoking may exceed the capacity of the oxidant defense system, resulting in oxidative damage to selected proteins, lipids, and DNA [4, 5, 12, 24]. Chelchowska et al. reported that MDA was significantly higher in the cord blood of newborns of smoking mothers [10]. We found that TOS levels were significantly higher in the active and passive smoker groups than in the controls.

The body, on account of its susceptibility to oxidative insult, is naturally provided with an efficient antioxidant system. A series of enzymes, vitamins, and other antioxidants act as scavenging systems. The enzymes superoxide dismutase and glutathione peroxidase and GST are the first line of defense against reactive oxygen species and are generally referred to as primary antioxidants [25]. In this study, we studied the nonenzymatic antioxidant system. Previous studies state that active and passive maternal smoking convincingly and significantly induces oxidative stress for the mothers and also for the neonate [3, 6, 18]. The present study demonstrated that placental tissues were affected by major oxidative stress in the active or passive smoker. The defense system may not counter oxidant stress, and oxidant/antioxidant status shifts to the oxidant side. Overall, therefore, it would seem that the onset of the full maternal blood oxidative status is associated with an increase in placental tissue oxidative stress in pregnancies.

A variety of oxidants and free radicals, resulting from cigarette smoke, are well documented to initiate or promote oxidative damage in peripheral and cord blood [3, 6, 25]. Free radicals are capable of directly and indirectly inducing oxidative stress in the body. Although the underlying mechanisms involved in the pathologies associated with smoking are still arguable, free radical-induced oxidative damage has been suggested to play a major role in the pathogenesis of numerous smoking-related disorders [30]. Bolisetty et al. reported that exposed infants have significantly lower antioxidant vitamins than do those not exposed [8]. They proposed that one consequence of the lower antioxidant vitamin levels in exposed infants is oxidative stress. Maternal smoking has been associated with numerous adverse pregnancy outcomes, including lipid hydroperoxides and oxidative stress [32]. Similarly, in infants exposed to passive smoking, several components of the antioxidant defense system have been reported to be impaired as compared with those not exposed [3, 4]. In addition, fetuses that have been exposed without regard to the mother's active or passive smoking have increased oxidative status [6, 33].

The potential damage that can be caused by free radicals is normally minimized by antioxidant systems [18]. In passive smoking infants, several components of the antioxidant defense system have been reported to be

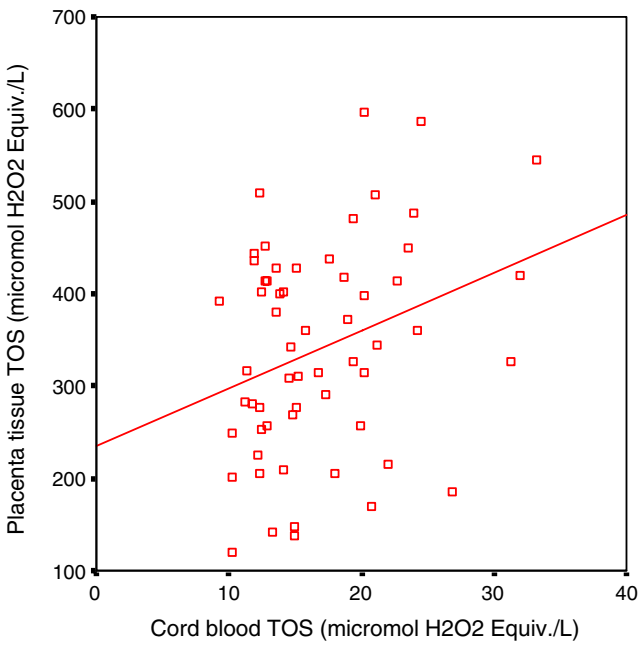

Fig. 4 Correlation graphic of TOS levels in placental tissue and cord blood ($r=0.304$, $P<0.016$)

impaired as compared with those not exposed to smoking [18, 19]. The previous studies that active and passive maternal smoking convincingly and significantly induces oxidative stress and for the mothers and also for the neonate [3, 6, 18]. Chelchowska et al. reported that TAC was significantly decreased in the cord blood of newborns of smoking mothers [10]. Fayol et al. demonstrated that TAC was low in the infant cord blood of passive smoking mothers, but this was not the case in the infants of active smokers [18]. In the present study, placental tissue TAC levels were significantly lower in the mothers who were active or passive smokers. However, peripheral and cord blood TAC levels were not significantly different in the mothers who were passive smokers. Our previous study showed that the mothers' peripheral blood TAC levels were lower in passive smokers than they were in non-smokers [6]. In that study the subjects were exposed to 6–25 cigarettes/day, and the mean age of the mothers who were passive smokers was 25.6±5.4 years while that of the controls was 24.7±4 years. Differences may be due to several factors, including maternal age, pregnancy status, body mass index, or number of cigarettes exposed to per day [21, 33].

In addition to these oxidative markers, OSI, which is a cumulative marker of both oxidative and antioxidative power [6], was significantly increased in the placental tissue of active and passive smokers. These results are the first on placental tissue. According to the data obtained in the present study, increased TOS levels, decreased TAC, cumulatively increased OSI and the other antioxidants together with the routine clinical parameters may implicate the presence of oxidative stress in the placenta subsequently in fetuses. These findings supported our previous data [3, 6]. In addition, decreased lipophilic antioxidants may play a role in the pathogenesis of atherosclerosis in the fetuses of mothers who are active or passive smokers through increased susceptibility to lipid peroxidation in utero.

In conclusion, active or passive maternal smoking is associated with important alterations in oxidant and antioxidant balance in placental tissue and causes potent oxidative stress.

Acknowledgements We are most grateful to the technical staff of the Laboratory and Delivery Unit at Sanliurfa Women's Hospital and Harran University Medical Faculty Research Hospital Biochemistry Department for their assistance in conducting this study.

Declaration of competing ınterests We wish to disclose a competing interest(s) such as those defined above or others that may be perceived to influence the results and discussion reported in this paper.

Institutional competing ınterests We aware that our academic institution or employment has not a financial interest in or a financial conflict with the subject matter or materials discussed in this manuscript.

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