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ORIGINAL PAPER

# Maternal active or passive smoking causes oxidative stress in cord blood

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**Abstract** The aim of this study was to assess the influence of active and passive maternal smoking on cord blood total oxidant/antioxidant status at term. The levels of cord blood catalase (CAT), paraoxonase 1 (PON1), ceruloplasmin, total thiol and lipid hydroperoxide (LOOH), total antioxidant capacity (TAC), total oxidant status (TOS) and the oxidative stress index (OSI) were measured in samples of fetal cord blood serum from 29 nonsmokers who were not exposed to active or passive smoke, 30 passive smokers and 21 active smokers. The gestation period of all pregnancies was between 37 and 40 weeks, the pregnancies were uncomplicated and the infants were delivered vaginally. The weights of infants borne to the active smokers were significantly ($P<0.01$) lower than those borne to the controls. Significantly lower concentrations of CAT, PON1 and TAC were found in the cord blood of the smokers than in that of the nonsmokers ($P<0.018$). The cord blood levels of LOOH and TOS and OSI were significantly higher in the active and passive smokers than in the controls ($P<0.01$). A significant positive correlation was found between maternal tobacco exposure and cord blood OSI ($P<0.001$). Active or passive maternal smoking is associated with important alterations in the balance of oxidants and antioxidants in fetal cord blood and causes potent oxidative stress.

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**Abbreviations**

| | |
|---|---|
| CAT | catalase |
| LOOH | lipid hydroperoxide |
| PON1 | paraoxonase 1 |
| OSI | oxidative stress index |
| TAC | total antioxidant capacity |
| TOS | total oxidant status |

## Introduction

Exposure to cigarette smoke in utero is associated with numerous adverse pregnancy outcomes, including intrauterine growth retardation and fetal death [32, 39, 40]. Several mechanisms have been postulated to explain such effects. Cigarette smoke contains an abundance of compounds emitted in gases and condensed tar particles, many of which are oxidants and pro-oxidants capable of producing reactive oxygen species [2]. The enhanced production of reactive oxygen species by smoke is related to increased free radical production, antioxidant depletion and oxidative stress [4, 5, 20, 30, 34], all of which can result in the oxidation of lipids, induction of DNA single-strand breakage, inactivation of certain proteins and the disruption of biological membranes [13, 31]. One possibility given credence by several in vitro studies [21, 23, 33, 36, 41] is that cigarette smoke, rich in free radicals and oxidizing species, depletes plasma antioxidants [8]. Cigarette smoking causes oxidative stress in pregnant women and may have a similar effect in fetuses. We previously reported that plasma levels of total antioxidant capacity (TAC) and oxidants in infants and their

mothers at 6–28 weeks are modified by passive smoking [4, 5]. In the present study, we evaluated the effect of active and passive smoking by the mother throughout pregnancy on the levels of antioxidant and oxidant status in umbilical cord blood serum collected at term.

## Subjects and methods

### Subjects

Cord blood samples were obtained from 80 women at delivery following a pregnancy of 37–40 weeks. The study included a consecutive series of 21 active smokers who smoked 4–15 cigarettes per day throughout their pregnancy (median: six cigarettes per day), a series of 30 passive smokers who passively smoked 5–25 cigarettes per day (median: 11 cigarettes per day) and a series of 30 nonsmokers who had never been exposed to active or passive smoking.

The women were 20–38 years of age (median: 29 years), had no medical history and attended the Sanliurfa Women's Hospital. A brief history was taken, and a complete physical examination was carried out. 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 and their mother were excluded from the study. All subjects were of similar socio-economic status and 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 Sanliurfa Women's Hospital and Sanliurfa Children's Hospital ethics committees approved the collection of umbilical cord blood serum for research. Both departments are conducting ongoing research projects on cord blood. The mothers were fully informed about the aim of the investigation and, following their consent to be involved in the study, 3–4 ml of cord blood was aspirated from the vein into syringes, and serum then separated from the cells by centrifugation at 1500 *g* for 10 min. All samples were stored at −80°C without preservative until assayed.

### Analytical methods

Catalase (CAT) activity was assayed as described by Goth [22]. Paraoxonase 1 (PON1) activities were measured using phenylacetate substrate [14] and were expressed as kilo units per liter (kU/l) serum. Serum thiol (total-SH group) content was measured using dithionitrobenzoic acid (DTNB) [27]. Total oxidant status (TOS), TAC and ceruloplasmin levels were measured by Erel's methods [17–19]. These methods are automated and colorimetric. 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-sulfonic acid) (ABTS) radical cation by antioxidants [9]. The results were expressed in micromoles $H_2O_2$/l and millimoles Trolox equivalents per liter, respectively, and the precision of this assay is excellent and lower than 3% [1, 26]. Erel's ceruloplasmin method is based on the oxidation of ferrous ion to ferric ion via ceruloplasmin ferroxidase activity [16] and was expressed as grams per liter serum. Lipid hydroperoxide (LOOH) concentrations of serum were measured using the automated ferric-xylenol orange method [3]. The percentage of TOS level to TAC level was regarded as the oxidative stress index (OSI) [6, 24]. To perform the calculation, the unit of TAC (mmol Trolox equivalent/l) was changed to .mole Trolox equivalent per liter, and the OSI value was calculated as follows: OSI= [(total oxidant status, .mol/l)/(TAC, .mol Trolox equivalent/l) × 100] [4, 25]. Uric acid concentrations were measured using a commercial kit (Abbott).

### Statistical analyses

The variables were checked for homogeneity of variance using the Levene statistic. Differences in the 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 significance of the Levene statistic was higher than 0.05. If the data variances were non-normally distributed, between-group differences were analyzed by the Kruskal-Wallis one-way analysis of variance by ranks. The data were expressed as mean ± standard deviation, and differences were considered to be statistically significant at $P<0.05$. Statistical analyses were performed by using SPSS for Windows, Release 11.5 (SPSS, Chicago, Ill.).

## Results

We found no significant differences between the newborns of the three groups in terms of male/female distribution, mean gestational age or length. The body weights of infants born to active smokers were lower than those born to the control subjects (Table 1). Cord blood levels of CAT, PON1, ceruloplasmin, uric acid, total thiol (total-SH group), TAC, lipid hydroperoxide, TOS and OSI are given in Table 2. CAT and PON1 concentrations were signifi-

**Table 1** Maternal age and gestational age and birth weight of newborn according to smoke exposure

| | Active smoker ($n$=21) | Passive smoker ($n$=30) | Controls ($n$=29) | $P$* |
|---|---|---|---|---|
| Maternal age (years) | 29.9±6.4[a] | 30.3±7.1 | 29.7±6.8 | 0.639 |
| Gestational age (weeks) | 38.6±1.6 | 39.7±1.7 | 39.2±1.5 | 0.698 |
| Birth weight (g) | 3.020±690 | 3.200±630 | 3.25±590 | 0.031[b] |
| Cigarettes per day | 6±4 | 11±6 | – | – |

*One-way ANOVA with the Tukey's honestly significant difference multiple comparison test.
[a] Data are given as the mean ± standard deviation
[b] The difference is between active smoker and controls.

cantly lower in the active smokers than in the passive smokers and controls ($P$<0.017); uric acid and ceruloplasmin concentrations were slightly higher in the active smokers than in the passive smokers and controls ($P$> 0.05); total thiol concentrations were significantly lower the active and passive smokers than in the controls ($P$=0.005); TAC levels were significantly lower in the active smokers than in the passive smokers and controls ($P$<0.017) and significantly lower in the passive smokers than in the controls. Conversely, LOOH levels were significantly higher in the cord blood of the active and passive smokers than in the controls, and TOS and OSI levels were significantly higher in the active smokers than in the passive smokers and controls ($P$=0.002). These levels were also significantly higher in the passive smoker s than in the controls. A significant positive ($P$<0.001) correlation was found between maternal cigarette exposure and cord blood OSI levels.

## Discussion

The major results of the present study are that fetal TOS is affected by active or passive exposure to cigarette smoke during pregnancy, namely TOS increased in both groups. Interestingly, CAT and PON1 levels were lower in the active and passive smokers than in the controls, while ceruloplasmin and uric acid concentrations did not differ between groups. These results indicate that the fetuses of the active and passive smokers were under potent oxidative stress. To the best of our knowledge, this is the first report showing low CAT, PON1, total thiol and TAC levels and high LOOH, TOS and OSI levels in the cord blood of fetuses in active and passive smokers.

A wide variety of oxidants and free radicals that result from cigarette smoke have been well documented to initiate or promote oxidative damage and lead to various degenerative pulmonary and cardiovascular diseases as well as cancers [28, 37]. Although the underlying mechanisms involved in the pathologies associated with smoking are still open to argument, it has been suggested that free radical-induced oxidative damage plays a major role in the pathogenesis of numerous smoking-related disorders [35]. Free radicals are capable of directly and indirectly inducing oxidative stress in the body. Bolisetty et al. reported that infants exposed to free radicals have significantly lower antioxidant vitamins than those who have not been exposed [7]. They also proposed that one consequence of the lower antioxidant vitamin levels in exposed infants is oxidative stress. 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 [4, 5, 30]. Similarly, maternal smoking has been associated with numerous adverse pregnancy outcomes, including lipid hydroperoxides and oxidative stress [23].

**Table 2** Effect of smoke exposure on cord blood oxidative and antioxidative parameters

| Antioxidative parameter[a] | Active smoker ($n$=21) | Passive smoker ($n$=30) | Control ($n$=29) | $P$* | Post Hoc[b] |
|---|---|---|---|---|---|
| Catalase (kU/l) | 29. 9±22.6[e] | 60.8±17. 9 | 58.2±19.5 | 0.017[c] | AS&PS, S&C |
| PON1 activity (kU/l) | 59.6±25.4 | 111.4±31.7 | 117.6±39.1 | 0.005[d] | AS&C, PS&C |
| Ceruloplasmin (g/l) | 0.318±0.088 | 0.305±0.067 | 0..314±0.065 | 0.597[d] | – |
| Uric acid (mmol/l) | 0.33±0.09 | 0.27±0.09 | 0.25±0.08 | 0.244[d] | – |
| Total-SH group (mmol/l) | 0.241±0.035 | 0.259±0.032 | 0.317±0.133 | <0.001[c] | AS&C, PS&C |
| TAC (mmol Trolox equivalent/l) | 1.46±0.19 | 1.53±0.22 | 1.59±0.08 | <0.001[c] | All |
| LOOH (μmol $H_2O_2$ equivalent/l) | 7.1±1.5 | 5.7±1.2 | 5.1±1.1 | 0.002[d] | AS&C, PS&C |
| TOS (μmol $H_2O_2$ equivalent/l) | 12.6±3.6 | 9.1±3.3 | 6.8±2.1 | 0.002[d] | All |
| OSI (arbitrary unit) | 8.4±2.1 | 6.1±2.4 | 4.3±2.1 | <0.001[c] | All |

*Significant difference from between marked groups ($P$<0.05).
[a] PON1, Paraoxonase 1, TAC, total antioxidant capacity; LOOH, lipid hyropeoxide; TOS, total oxidant status; OSI, oxidative stress index.
[b] AS, active smoker; PS, passive smoker; C, control
[c] Kruskal-Wallis test
[d] One-way ANOVA with Tukey's honestly significant difference multiple comparison test
[e] Data are given as mean ± standard deviation

The body, on account of its susceptibility to oxidative insult, is naturally provided with an efficient antioxidant system. A series of enzymes also act as scavenging systems, including superoxide dismutase (SOD) and glutathione peroxidase (GPX). These enzymes are the first line of defense against reactive oxygen species and are generally referred to as primary antioxidants. The level of CAT, which is a peroxisomal hydrogen peroxide-consuming enzyme, increases in chronic smokers, whereas the levels of several antioxidant enzymes, such as CuZnSOD, glutathione transferase (GST) and GPX, appear to decline in smokers with long smoking histories [29]. In this study, we found that serum CAT levels were lower in active smokers than in the passive smokers and the controls.

We found that basal/salt-stimulated PON1 activities were significantly lower in patients who were active smokers than in passive smokers and the controls, while LOOH and TOS levels were significantly higher. To our knowledge, this is the first paper reporting the PON1 activities in the cord blood of infants of mothers who are active or passive smokers. According to the data obtained in the present study, increased LOOH and TOS levels, decreased PON1 activities and the presence of other antioxidants together with the routine clinical parameters may implicate the presence of oxidative stress in fetuses. In addition, decreased lipophilic antioxidants and PON1 activities may play a role in the pathogenesis of atherosclerosis in infants of active smoking mothers through an increased susceptibility to lipid peroxidation in utero.

Ceruloplasmin is a copper-containing glucoprotein with multiple physiological functions, including ferroxidase and oxidase activity. It is induced by inflammatory processes, air pollution, and cigarette smoking [2, 12, 38]. The level of ceruloplasmin has been found to be significantly increased in neonates whose mothers were active or passive smokers [12]. We found that ceruloplasmin levels did not differ significantly between each of the three groups. Fayol et al. reported that cord blood antioxidant parameters strongly correlated with maternal antioxidant status, with the exception of ceruloplasmin [20]. They also stated that an altered neonatal ceruloplasmin concentration may reflect the effect of cigarette exposure on the antioxidant system of the neonate rather than transplacental transfer of maternal ceroloplasmin. However, wee did not find any correlation in the present study. Further investigations are needed to address this issue.

Uric acid is a well-known low-molecular-weight water-soluble plasma antioxidant [17, 34]. Several clinical studies in humans have demonstrated increased uric acid production as a result of oxidative stress, such as that related to smoking [15]. Fayol et al. reported that the uric acid levels in cord blood of passive smoking infants was significantly higher than that in the cord blood of the controls [20]. However, while we found that plasma uric acid levels were slightly higher in the active smokers than in the passive smokers and the control, the difference was not statistically significant ($P>0.05$).

The potential damage that can be caused by free radicals is normally minimized by the antioxidant systems. In passive smoking infants, several components of the antioxidant defense system have been reported to be impaired as compared with those of infants not exposed to smoking [24]. Chelchowska et al. [10] reported that the level of TAC was significantly decreased in the cord blood of newborns of smoking mothers. Fayol et al. [20] demonstrated that the TAC level was low in the infant cord blood of passive smoking mothers but that this was not the case in the infants of active smokers. In this study, we found that total thiol and TAC levels were lower in active and passive smokers than in the controls.

The values of lipid peroxidation products can be used as an index of oxygen free radical generation. The measurement of LOOH and TOS provides a sensitive index of lipid peroxidation and oxidative stress [3, 19]. 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 [11, 37, 42]. Chelchowska et al. reported that the level of malondialdehyde was significantly higher in the cord blood of newborns of smoking mothers. We found that LOOH and TOS levels were significantly higher in the active and passive smokers. In addition to these oxidative markers, OSI, which is a marker of both oxidative and antioxidative power, was significantly increased in the cord blood of active and passive smokers. These results are the first to be published on fetal cord blood.

In conclusion, active or passive maternal smoking is associated with important alterations in the oxidant and antioxidant balance in fetal cord blood and causes potent oxidative stress.

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