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ORIGINAL PAPER

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Increased oxidative stress in infants exposed to passive smoking

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Abstract The purpose of this study was to assess the effect of passive cigarette smoking on the oxidative and anti-oxidative status of plasma in infants. Eighty-four infants aged 6–28 weeks were divided into two groups: the study group included infants who had been exposed to passive smoking via at least five cigarettes per day for at least the past 6 weeks at home, while the control group included infants who had never been exposed to passive smoking. The antioxidative status of plasma was assessed by the measurement of individual antioxidant components: vitamin C, albumin, bilirubin, uric acid, thiol contents and total antioxidant capacity (TAC 1 and TAC 2). Oxidative status was assessed by the determination of total peroxide levels and the oxidative stress index (OSI 1 and OSI 2). Plasma vitamin C, thiol concentration and TAC 1 and TAC 2 levels were significantly lower, whereas plasma total peroxide levels and OSI 1 and OSI 2 were significantly higher, in passive smoking infants than in the controls ($P < 0.01$). We conclude that passive smoking has a negative impact on numerous parts of the antioxidant defence system in infants, and exposes them to potent oxidative stress.

Keywords Antioxidants · Infant · Lipid peroxides · Oxidative stress · Passive smoking

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Introduction

Cigarette smoke contains numerous 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 can produce a condition of oxidative stress that can result in the oxidation of lipids, induction of DNA single-strand breakage, inactivation of certain proteins, and the disruption of biological membranes [8, 21]. Increased oxidative stress has been suggested to play a major role in the pathogenesis of several smoking-related diseases, such as cancer, cardiovascular diseases, and oral diseases [23, 28, 32]. Precisely how cigarette smoking contributes to atherogenesis remains obscure. One possibility given credence by several in vitro studies [11, 13, 22, 29, 36] is that cigarette smoke, rich in free radicals and oxidising species, depletes plasma antioxidants [4]. On the other hand, these dangerous molecules are also taken from exogenous sources, such as cigarette smoke. There is evidence that smoking is related to increased free-radical production, antioxidant depletion and oxidative stress [25].

Under some conditions, oxidants increase and antioxidants decrease and antioxidative mechanisms may be insufficient to prevent oxidative damage completely. Consequently, oxidative stress develops [12]. Although smoking is recognised to be a significant health problem in adult subjects [7], the health consequences of passive smoking on infants who have a short-term passive smoking history are understood less. To the best of our knowledge, all the published studies related to the oxidative effects of passive smoking are about children [20] and adults [35] rather than infants. In this study, we investigated the oxidative and antioxidative status of infants exposed to passive smoking and compared it with that of infants not exposed.

Subjects and methods

Subjects

The study group consisted of 52 infants (30 male, 22 female), 6–28 weeks old (mean 14.9 ± 0.9 weeks). These subjects had been exposed to at least five cigarettes per day (range 5–25 cigarettes/day and mean 10 ± 3 cigarettes/day) for at least the last 6 weeks at home, but their mothers were not active smokers. All subjects were breast fed, and none had taken any antioxidant medications (vitamin C, vitamin E, selenium, etc.) or drunk fruit juice prior to or during the study.

The control group consisted of 32 infants (19 male, 13 female), 6–28 weeks old (mean 14.9 ± 1.1 weeks). These subjects had never been exposed to passive smoking. A brief history was taken, a complete physical examination was performed, and blood samples for complete blood count and renal and liver function tests were obtained for each individual in the study and control groups. Subjects with any signs or symptoms of any acute or chronic illness or with abnormal biochemical test results were excluded from the study. The subjects' parents were of similar socioeconomic status, living in a rural region. The mothers had been fully informed about the aim of the investigation and gave their consent to be involved in the study.

Methods

Blood samples were withdrawn into heparinised tubes, and plasma was separated from the cells by centrifugation at 3,000 rpm for 10 min. The plasma samples were stored at −80°C until required for analysis. The plasma was analysed for total antioxidant capacity 1 and 2 (TAC 1, TAC 2), thiol, ascorbic acid, uric acid, bilirubin, total protein and albumin.

TAC 1 and TAC 2 levels were determined by two different novel automated methods developed by Erel [9, 10]. In the first method (TAC 1), hydroxyl radical, the most potent biological radical, is produced by the Fenton reaction and reacts with the colourless substrate *o*-dianisidine to produce dianisyl radical, which is bright yellowish-brown. The second method (TAC 2) is based on the bleaching of the characteristic colour of a more stable 2,2′-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical cation by antioxidants [5]. The assay results are expressed in millimole Trolox equivalent units per litre, and the precision of this assay is excellent—lower than 3% [1, 15, 16]. Total peroxide concentrations of the plasma were determined by the FOX2 method [24] with minor modifications [14]. The FOX2 test system is based on the oxidation of ferrous iron to ferric iron by the various types of peroxides contained in the plasma samples, in the presence of xylenol orange, which produces a coloured ferric–xylenol orange complex whose absorbance can be measured. Aliquots (200 μl) of plasma were mixed with 1.8 ml of FOX2 reagent.

After incubation at room temperature for 30 min, the vials were centrifuged at 12,000 *g* for 10 min. The absorbance of the supernatant was then determined at 560 nm. The total peroxide content of the plasma samples was determined as a function of the difference in absorbance between the test and blank samples, with a solution of H_2O_2 used as standard. The coefficient of variation for individual plasma samples was less than 5%. Plasma total protein, albumin, uric acid and bilirubin levels were measured with commercial kits (Abbott). Vitamin C concentration was measured by the FRASC method [3]. The percent ratio of the total peroxide to the TAC 1 and TAC 2 gave the oxidative stress index 1 (OSI 1) and OSI 2, an indicator of the degree of oxidative stress [14, 33, 34].

The data obtained from the study and control groups were compared using Student's *t*-test and chi-square test. Differences were considered statistically significant at $P < 0.05$.

Results

We found no significant differences between the groups with regard to male/female distribution, mean age, height or head circumference. Body weights tended to be lower in the study group than in the control group, but this difference was not statistically significant (Table 1).

Plasma total protein, albumin and vitamin C concentrations, and total thiol (total -SH group), TAC 1 and TAC 2 levels are shown in Table 2. Vitamin C concentration of the plasma was significantly lower in the study group than in the control group ($P < 0.001$).

Table 1 Comparison of age, height, body weight and head circumference of passive smoking infants (study group) and control group. Data given as mean ± SD

Parameter	Study group ($n = 52$)	Control group ($n = 32$)	*P*
Age (weeks)	14.9 ± 0.9	14.9 ± 1.1	0.702[a]
Height (cm)	60.6 ± 5.8	60.7 ± 5.2	0.697[a]
Body weight (kg)	6.1 ± 1.7	6.5 ± 1.5	0.618[a]
Head circumference (cm)	40.2 ± 2.4	40.1 ± 1.9	0.722[a]
Gender (male/female)	30/22	19/13	0.215[b]
Number of family individuals	7.6 ± 2	6.3 ± 1.8	
Cigarettes per day	10 ± 3	–	
Number of smokers	1.4 ± 0.6	–	

[a]Student's *t*-test
[b]Chi-square test

Table 2 Comparison of oxidative and antioxidative parameters of plasma of study group and control group. Data given as mean ± SD

Parameter	Study group ($n = 52$)	Control group ($n = 32$)	P^a
Uric acid (mmol/l)	0.14 ± 0.011	0.17 ± 0.018	0.257
Total bilirubin (μmol/l)	6.84 ± 0.43	8.55 ± 2.05	0.076
Total protein (g/l)	58 ± 11.8	62 ± 12.7	0.025
Albumin (g/l)	37 ± 4.6	40 ± 4.5	< 0.001
TAC 1 (mmol Trolox equiv./l)	1.17 ± 0.06	1.59 ± 0.12	0.002
TAC 2 (mmol Trolox equiv./l)	1.42 ± 0.22	1.66 ± 0.18	0.003
Total -SH group (mmol/l)	0.42 ± 0.01	0.46 ± 0.01	0.009
Vitamin C (μmol/l)	89.3 ± 9.4	105.8 ± 10.2	< 0.001
Total peroxide (μmol H_2O_2/l)	24.6 ± 8.6	21.9 ± 5.5	0.002
OSI 1 (arbitrary unit)	2.1 ± 0.74	1.38 ± 0.45	0.007
OSI 2 (arbitrary unit)	1.73 ± 0.41	1.31 ± 0.37	0.001

[a]Student's *t*-test

Mean (SD) values were 89.3 (9.4) μmol/l and 105.8 (10.2) μmol/l, respectively. Thiol concentration was significantly lower in the study group than in the control group ($P = 0.011$). Mean (SD) values were 0.42 (0.01) mmol/l and 0.46 (0.01) (10.2) mmol/l, respectively. TAC 1 was significantly lower in the study group than in the control group ($P = 0.002$). Mean (SD) values of TAC 1 were 1.17 (0.12) mmol Trolox equiv./l and 1.59 (0.06) mmol Trolox equiv./l and TAC 2 were 1.42 (0.22) mmol Trolox equiv./l and 1.66 (0.18) mmol Trolox equiv./l, respectively. Conversely, the mean (SD) total peroxide level was significantly higher in the study group, 24.6 (8.6) μmol H_2O_2/l, than in the control group, 21.9 (5.5) μmol H_2O_2/l ($P = 0.02$). The mean (SD) OSI value was significantly higher in the study group, 2.1 (0.74), than in the control group, 1.38 (0.45) ($P = 0.007$).

Discussion

In the present study we found that the oxidative/antioxidative balance shifted towards the oxidative side, namely oxidative stress was present in study group compared to the control group. To the best of our knowledge, all the published studies related to the oxidative effects of passive smoking are about children [20] and adults [35]; this is the first report showing an association between low antioxidant capacity and passive smoking in infants.

Cigarette smoking has been implicated as a significant risk factor for the establishment and progression of several diseases. 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 [27]. Free radicals are capable of directly and indirectly inducing oxidative stress in the body. The major cause of chronic oxidative stress in humans is exposure to free radicals in cigarette smoke [17]. Free radicals originating from cigarette smoke are considered one of the most important causes of atherosclerosis and cancer [26].

Plasma has various antioxidant molecules. Albumin, uric acid, bilirubin and ascorbic acid are the major antioxidant components of plasma. TAC represents practically all of them. Albumin consists of about half of the total antioxidant capacity of plasma [9, 10]. Plasma thiol contents originate from albumin. In chronic inflammation it is reduced. Smoking leads to an inflammation process. We found that plasma albumin and thiol levels in the study group were significantly lower than those in the control group.

Clinical studies on smokers have indicated adverse effects of smoking on ascorbic acid metabolism, and several investigators have reported that plasma ascorbic acid concentrations are lower in cigarette smokers than in non-smokers [18, 31]. Lower plasma ascorbic acid concentrations in smokers may be due to several factors, including decreased ascorbic acid consumption, impaired ascorbic acid absorption, or an increased turnover of ascorbic acid [3]. We found that the low plasma ascorbic acid concentrations observed in the study group were due to the oxidative stress imposed by passive smoking. In fact, it has been suggested that serum ascorbic acid was lower in smoking than in non-smoking teenage girls [19]. We also found statistically significant lower plasma ascorbate levels in the study group than in the control group ($P < 0.05$). According to the literature, this is the first report on infants.

Uric acid is another well-known low molecular weight water-soluble plasma antioxidant [9, 25]. We observed that plasma uric acid levels were lower in the study group than in the control group, but the difference was not statistically significant ($P > 0.05$). It has been shown that bilirubin is a potent endogenous antioxidant molecule [21, 22]. It has been also shown that bilirubin concentration is lower in smokers than in non-smokers [30]. In this study, we determined lower serum bilirubin levels in the infants exposed to passive smoking than in those in the control group. The decrease in serum bilirubin concentration led to a weakness of serum TAC. TAC 1 and TAC 2 levels were lower in the study group than in the control group.

Plasma total peroxide levels in infants were increased by passive smoking. It has been reported that tobacco

smoke is a rich source of oxidants and reactive oxygen species. It has been argued that the increased production of reactive oxygen species associated with smoking may exceed the capacity of the oxidant defence system, resulting in oxidative damage to selected proteins, lipids, and DNA [6, 37]. We found that the OSIs were significantly higher in the study group. These results are also the first on infants.

In conclusion, passive smoking has a negative effect on numerous parts of the antioxidant defence system in infants, based on a higher oxidative stress index, lower TAC levels, and lower plasma vitamin C and thiol concentrations.

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