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Association of Smoking with Total Oxidant and Antioxidant Levels in Breast Milk

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Abstract

Background: Breast milk is a natural food that contains all the fluids, energy, and nutrients necessary for the optimum growth and development of newborns. Smoking is a public health problem that has harmful effects on the mother and baby. This study aimed to examine the association of exposure to smoking with total oxidant status (TOS) and total antioxidant status (TAS) in breast milk.
Methods: Healthy mothers without any health problems during their pregnancy and lactation periods were selected as subjects. Eighty-eight milk samples (44 in the smoking group and 44 in the nonsmoking group) were examined. TOS and TAS were analyzed using Rel Assay commercial kits.
Results: TAS level was significantly lower in the smoking group than in the nonsmoking group ($p < 0.05$). TOS level was higher in the smoking group than in the nonsmoking group, although the difference was not statistically significant ($p > 0.05$). Oxidative stress index (OSI) was significantly higher in the smoking group than in the nonsmoking group ($p < 0.05$).
Conclusions: Exposure to smoking was associated with low TAS and high OSI in breast milk.

Keywords: cigarette smoke, human breast milk, oxidative stress, total antioxidant level, total oxidant level

INTRODUCTION

Owing to its excellent composition (such as carbohydrate, fat, protein, vitamins, and minerals), breast milk is the best food for newborns and babies. Breast milk volume is highly variable, and its content is affected by the mother's diet or stored nutrients.[1] Breast milk plays an important role in transferring the antibodies that the baby needs from the mother to the baby.[2] Colostrum is valuable for newborns because of its immune molecules and is abundant in proteins, immunoglobulins, cytokines, and leukocytes.[3] After a few weeks, the content of colostrum changes and it transitions to mature milk containing numerous bioactive components, such as essential nutrients, hormones, growth factors, and enzymes.[2,4] The quality of breast milk is directly related to the mother's health. Essential nutrients passing from the mother into the milk are affected by maternal nutrition and lifestyle.[1]

Cigarettes have various oxidants, particularly nitric oxide (NO) and nitrogen dioxide (NO_2) in the gas phase. Particles in the gas phase are short lived. In the tar phase, water-soluble radicals are produced by the hydroquinone–quinone cycle.[5] These oxidants derived from tobacco smoke can damage biomolecules such as proteins, lipids, and DNA, which are the basic structural and functional molecules of the cell.[6] Certain substances in cigarettes and smoke have various harmful effects on an organism, including mutagenic/carcinogenic effects, irritation, and tumor acceleration.[7] These effects also cause many diseases, such as cardiovascular diseases,[8] periodontal diseases,[9] inflammatory diseases,[10] neurodegenerative diseases,[11] inflammatory bowel disease,[12] chronic obstructive pulmonary disease,[13] and lung cancer.[14] Exposure to cigarettes alters the total oxidant status (TOS), total antioxidant status (TAS), and vitamin C and E levels.[15] Overproduction of oxidant molecules can suppress the protective effect of breast milk. Smoking reduces the protective properties of milk by causing negative changes in milk composition and negatively affecting the health of the baby. Nicotine levels in breast milk are three times higher than those in the plasma of women who smoke.[16] Therefore, this study aimed to show how smoking exposure affects TAS and TOS in breast milk.

METHODS

Ethical approval

Ethics committee approval was obtained (2017/77 e.c. approval code) from Recep Tayyip Erdoğan University in Turkey Non-Interventional Clinical Research Ethics Committee. Breast milk samples were collected from puerperant women from the patient portfolio of the Family Health Centers of Erzurum Provincial Health Directorate. This study was carried out in two phases, namely, questionnaire assessment and biochemical analysis.

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Participants

G* Power 3.1.9.2 program was used to calculate the number of samples. Power analysis revealed effect size: 0.80, margin of error (α): 0.05, and 95% power. On the basis of these results, the minimum number of samples to be collected was set as 35 for each of the experimental and control groups. Eighty-eight mothers participated in this study. According to their responses, two groups were established, namely, exposed to smoking (N = 44) and not exposed to smoking (N = 44). Individuals excluded from the study were those with hypertension, gestational diabetes, constipation, chronic kidney failure, severe anemia, and malignity.

Questionnaire application

Informed consent was obtained from the volunteer mothers between 1st and 6th months of lactation. Responses to the questions in the Interview Questionnaire Form (information on maternal health, pregnancy and baby, and smoking status) were recorded.

Biochemical analysis

No preservatives were used while collecting the breast milk samples. Approximately 10 mL of milk samples were collected from each mother and placed in falcon tubes. The milk samples were stored at −20 °C in the dark. TAS and TOS in milk samples were measured in one single batch using Abbott C16000 autoanalyzer at Recep Tayyip Erdoğan University Training and Research Hospital Biochemistry Laboratory, Turkey.

Measurement of total antioxidant status (TAS)

TAS in breast milk samples was measured by an autoanalyzer using the automated colorimetric method. The hydroxyl radical ($OH^{\cdot}$) produced by the Fenton reaction reacts with the colorless substrate O-dianisidine to produce the yellowish brown dianisyl radical. After the plasma sample is added, the oxidative reactions initiated by the hydroxyl radicals are suppressed by the antioxidant components of the plasma. Discoloration is prevented by antioxidants. In this way, the total antioxidant capacity of the plasma is measured. Trolox, a water-soluble analog of vitamin E, was used as the calibrator standard. The results were expressed as mmol Trolox equivalent/L.[17]

Measurement of total oxidant status (TOS)

TOS in milk samples was measured using the method of Erel (2005). İn the first step, oxidants in the sample oxidize the Fe^{+2}-o-dianisidine complex to Fe^{+3}. This oxidation is enhanced by glycerol molecules in the reaction medium. Ferric ion is generated as a result of the reaction and forms a colored complex with xylenol orange in an acidic environment. Color intensity was measured spectrophotometrically, and the total oxidant molecule level was determined. Calibration was conducted with hydrogen peroxide (H_2O_2). The results were expressed as micromolar H_2O_2 equivalent per liter (μmol H_2O_2 equivalent/L).[18]

Calculation of Oxidative Stress Index (OSI)

OSI is an indicator of oxidative stress. For the calculation of OSI in the samples, the units of TAS and TOS were equalized and expressed as the ratio of TOS to TAS in terms of percentage.[19] The results were expressed as arbitrary unit (AU).

$$OSI = \frac{TOS, \mu mol\ H2O2\ Equivalent/L}{TAS, \mu mol\ Trolox\ Equivalent/L} \times 100$$

Statistical analysis

Statistical analysis was conducted using the SPSS for Windows Version 17.0 package program. Nonparametric Mann–Whitney U test was used to evaluate the difference between groups. $p < 0.05$ was considered significant.

RESULTS

The demographic characteristics of the participating mothers in both groups showed similarities ($p > 0.05$) in terms of the mean age, weight, weight before pregnancy, total number of births, first gestational age, and gestational age of the mother and the mean birth weight of the baby (Table 1).

In Table 2, TAS levels are expressed in millimoles, TOS levels in micromoles, and OSI levels in arbitrary units. While the mean of TAS level was 0.678 (±0.534) in the group exposed to cigarettes, the mean of TAS level was found to be 1.404 (±1.472) in the group not exposed to cigarettes. The mean of TAS level is approximately two times higher in non-exposed to smoking. While the mean of TOS level was 3.686 (±1.749) in the group exposed to cigarettes, the mean of TOS level was found to be 3.387 (±1.314) in the group not exposed to cigarettes. The average of TOS levels is relatively higher in smokers. While the mean of OSI value was 1.178 (±1.258) in the group exposed to cigarettes, the mean of OSI value was found to be 0.761 (±1.187) in the group not exposed to smoking. The reason why the OSI index was lower in the non-smoker group is that TAS levels were higher in this group.

As shown in Table 3, TAS was higher in women not exposed to smoking than in those exposed to smoking ($p < 0.05$). No significant difference in TOS was observed between the two groups ($p > 0.05$). OSI was statistically significantly higher in the mothers exposed to smoking than in those not exposed to smoking ($p < 0.05$).

TABLE 1. Demographic characteristics of the participants

Variables	Exposed to smoking			Not exposed to smoking			*p*
	Range	Median	IQR	Range	Median	IQR	
Maternal age (year)	19–41	31.50	8	21–43	29.50	7	0.815
Maternal weight (kg)	47–100	65	15	52–105	69	17	0.732
Weight before pregnancy (kg)	46–100	64.50	19	48–92	64.50	16	0.764
Total births	1–9	3	2	1–6	2	2	0.079
First pregnancy age (year)	17–38	23	6	15–35	25	6	0.089
Gestational age (weeks)	33–41	39	2	32–42	38	2	0.105
Birth weight (g)	2,100–5,000	3,100	750	1,360–4,500	3,000	575	0.296

IQR: Interquartile range

TABLE 2. TAS, TOS, and OSI in the groups exposed and not exposed to smoking (N =44)

Group	Range	Mean	SD	Median	IQR
Exposed To Smoking					
TAS (mmol)	0.045–1.777	0.678	0.534	0.522	0.700
TOS (μmol)	1.530–9.400	3.686	1.749	3.199	2.350
OSI (AU)	0.100–5.770	1.178	1.258	0.619	1.400
Not exposed to smoking					
TAS (mmol)	0.074–5.689	1.404	1.472	0.923	1.349
TOS (μmol)	0.640–5.980	3.387	1.314	3.323	1.360
OSI (AU)	0.030–7.320	0.761	1.187	0.384	0.830

Values are presented as mean, standard deviation (SD), and IQR: Interquartile range.

TABLE 3. Comparison of TAS, TOS, and OSI between the two groups (Mann–Whitney U test) (N = 44)

Group	Median	IQR	*p*
TAS			
Exposed To Smoking	0.522	0.700	0.010
Not exposed to smoking	0.923	1.349	
TOS			
Exposed To Smoking	3.199	2.350	0.924
Not exposed to smoking	3.323	1.360	
OSI			
Exposed To Smoking	0.619	1.400	0.013
Not exposed to smoking	0.384	0.830	

IQR: Interquartile range

DISCUSSION

In this study, we aimed to reveal the relationship between exposure to smoking and breast milk TAS and TOS. Results showed that TAS of the milk from mothers exposed to smoking was lower than the other group. Plasma total antioxidant level decreases substantially in individuals who smoke.[15] Some studies revealed a similar result for breast milk. In one research testing the colostrum and mature milk samples of 30 women exposed to smoking at least five times a day during pregnancy and lactation and 29 women not exposed, the colostrum TAS was found to be significantly lower in the smoking group than in the other group.[20]

One study on the effects of maternal smoking exposure during pregnancy and lactation on colostrum found that breast milk TAS was significantly lower in smokers than in nonsmokers. Smoking during pregnancy and lactation decreases the antioxidant properties of colostrum.[21] Comparison was conducted on the breast milk samples of smoking and nonsmoking women in terms of vitamin E concentration in the third trimester of pregnancy and tocopherol concentration in the postpartum period, and the results showed that serum vitamin E concentration did not differ between the groups but vitamin E concentration in mature milk was significantly lower in smokers than in nonsmokers.[22] In another study, beta carotene concentrations in maternal blood and umbilical cord were found to be significantly higher in smoking mothers than in nonsmoking mothers.[23] Venous blood samples were collected from smokers and nonsmokers on the day they gave birth, and β-carotene, retinol, α-tocopherol, and cotinine levels were measured in milk and infant urine samples on the 7th day after birth. The results showed lower α-tocopherol levels in the milk of smoking mothers compared with that of nonsmokers. Maternal smoking may decrease vitamin E levels in milk because

the antioxidants are utilized to limit lipid peroxidation.[24] In a study measuring nesfatin-1, irisin, malondialdehyde (MDA) levels, and superoxide dismutase (SOD) activity in the milk of smoking and nonsmoking mothers, nesfatin-1 and MDA levels of the mothers who smoked were found to be higher than those of the nonsmoker group. In the same study, the breast milk SOD activity of smoking mothers was found to be lower than that of the control group.[25]

Karademirci *et al*. investigated the effects of long-term smoking on oxidative stress on 78 smokers and 82 individuals with no risk factors and found that serum TAS was significantly low in chronic smokers.[15] In another study on 20 smokers and 20 nonsmokers, the mean TAS was found to be low in the smoker group due to the numerous toxic substances in cigarettes and the severity of oxidative stress.[26] An investigation of the effect of smoking cessation on oxidative stress-related plasma components and enzyme activities among 1255 smokers and 524 healthy nonsmokers found that erythrocyte superoxide dismutase, catalase, and glutathione peroxidase enzyme activities were significantly low in smokers.[27] Our TAS results conformed with the previous studies. Smoking and exposure to smoking negatively affect and eventually reduce the level of antioxidant systems in tissues and breast milk.

In our study, TOS did not differ between the two groups. However, a significant difference in OSI (TOS/TAS ratio) was determined between the two groups. OSI was significantly higher in the breast milk of women with smoking exposure compared with that in the other group. Therefore, exposure to smoking increases oxidative stress in breast milk. In a previous work, many nonsmoking women exposed to second-hand tobacco smoke during lactation showed increased plasma oxidative stress and decreased antioxidant level.[28] Karademirci *et al*. evaluated the relationship of smoking with TAS, TOS, and OSI and reported that chronic smoking caused a significant increase in serum TOS level.[15] As a result, oxidative stress was high in the smoking group. Ermiş *et al*. investigated MDA level and SOD and glutathione peroxidase (GPx) activities in the serum and breast milk samples of 15 active-smoker, 22 passive-smoker, and 23 nonsmoker women.[29] Serum MDA and SOD displayed no difference, but GPx activities were significantly different between the groups. Among the milk samples of the three groups, the difference in MDA level and SOD activity was significant but that in GPx activity was not significant. They interpreted this result as human milk (even for passive smokers) is more vulnerable to oxidative stress and lipid peroxidation than serum samples. Another research revealed an increase in peripheral blood leukocytes and oxidant release among passive smokers.[30] Mahmood *et al*. found increased oxidant levels in individuals with exposure to smoking. Tobacco and certain ingredients in cigarettes deteriorate oxidative balance, increase oxidative stress, and eventually cause cell damage.[26]

Among patients with breast cancer, an increased plasma oxidative stress and decreased antioxidant level were found to be associated with exposure to smoking.[31] Another investigation found no difference in on TAS, TOS, OSI, and paraoxonase activity between smokers and nonsmokers.[32] Our results revealed significantly higher OSI in the group with smoking exposure compared with the control group; however, the difference for TOS was not statistically significant. This result was affected by the low TAS and/or the high ratio of TOS/TAS and indicated the increased levels of oxidants in the milk of mothers exposed to smoking.

CONCLUSIONS

In concordance with literature, this study shows that exposure to smoking adversely affects biochemical processes in breast milk and human tissues, resulting in reduced levels of antioxidants and increased levels of oxidants. As the essential nutrient for newborns, the content of breast milk is affected by maternal smoking exposure, which is an important risk factor for newborn health.

CONFLICT OF INTEREST

None declared.

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