Women's Health Bull. 2015 April; 2(2): e24973. DOI: 10.17795/whb-24973

Published online 2015 April 1. **Brief Report**

The Reduction of Maternal Milk Proteins in Mothers Exposed to Passive Smoking: A Prospective Cohort Study

Azar Shamsi [1,*]; Azam Baheiraei [2]; Shahnaz Khaghani [3]; Afshin Mohsenifar [4]; Anoshirvan Kazemnejad [5]

[1]Department of Maternal and Child Health, Tehran University of Medical Sciences, Tehran, IR Iran
[2]Department of Reproductive Health, Tehran University of Medical Sciences, Tehran, IR Iran
[3]Department of Medicine, Biochemistry Division, Tehran University of Medical Sciences, Tehran, IR Iran
[4]Department of Toxicology, Tarbiat Modares University, Tehran, IR Iran
[5]Department of Biostatistics, Tarbiat Modares University, Tehran, IR Iran

*Corresponding author: Azar Shamsi, Department of Maternal and Child Health, Tehran University of Medical Sciences, Tehran, IR Iran. Tel: +98-9121855437, Fax: +98-2188991694, E-mail: a.midwifery@gmail.com

Received: October 29, 2014; **Accepted:** January 10, 2015

Background: The number of cigarette smokers in people of all ages and the resulting second hand smokers are increasing worldwide. Smoking at home, work or in public places puts others at risk of exposure to second hand smoke.
Objectives: To study the effects of second-hand smoking on breast milk proteins.
Patients and Methods: This cohort study was conducted on 45 mothers exposed to second-hand smoke (cases) and 45 non-exposed post-partum mothers (controls) who attended health care centers. Milk samples were collected twice, (5-7 days and 4 months after delivery). Exposure was assessed through questionnaires which measured total levels of milk protein and albumin, and milk cotinine, a major metabolite of nicotine.
Results: Cotinine levels in the breast milk of mothers in the exposed group were significantly higher than non-exposed group at baseline and 4 months after delivery (P = 0.001). Milk protein profiles in the non-exposed group were significantly higher 5-7 days after delivery in the non-exposed group, but the albumin profile was not significantly different at 4 months post-partum (P = 0.004).
Conclusions: Second-hand smoke affects the levels of breast milk proteins that are essential for infant growth.

Keywords: Second Hand Smoke; Breast Feeding; Milk Proteins

1. Background

The number of cigarette smokers of all ages is increasing worldwide, and consequently, many people are second-hand smokers (1). Smoking at home, work or in public places puts others at risk of exposure to Second Hand Smoke (SHS) (2). While most countries have policies discouraging smoking in public places, exposure to cigarette smoke at home is not always avoidable (1). Based on previous studies, levels of nicotine and tar produced by cigarettes are three times higher in the blood of passive than active smokers. In addition, nicotine has been observed to pass through the breast milk of mothers to their infants (3, 4). Second-hand smoke exposure is present in 41.7% of homes and 50.6% of areas outside homes in Iran (5). Tobacco smoke exposure is evaluated by different methods, such as self-reported questionnaires and cotinine level measurements (6). Cotinine is a major metabolite of nicotine and has been shown to have longer half-life than nicotine (7). Cotinine could be measured in different parts of the infants' body such as hair, saliva, plasma and urine (8). Both active and second-hand smokes influence maternal and neonatal health. Neonates born to women who smoke may have lower birth weight, pre-term birth, and respiratory diseases (9). Smoker mothers tend breast feed less due to the effects of nicotine on dopamine and prolactin (10, 11). Furthermore, previous studies have shown that smoking may change the protein content of mothers' milk, which may affect the infant's well-being (12, 13).

2. Objectives

This study aimed to evaluate the effects of second-hand smoke on breast milk proteins.

3. Patients and Methods

This cohort study was conducted in Tehran in 2010. Similar to a study conducted by Agostoni et al. (14) considering 80% power for the study and anticipating 20% loss, 45 individuals were included in each group. Therefore, a total of 90 women were selected by simple random sampling among those who referred to health care centers 5-7 days after delivery. These women were assigned to the

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case (n = 45) or control (n = 45) groups, based on exposure to second-hand cigarette smoke. All subjects were asked to sign informed consents before participating in the study. Women with diseases affecting breast feeding, on special medications, exposed to substances other than cigarette smoke, and breastfeeding for less than 4 months were excluded from the study. Second hand exposure to cigarette smoke was evaluated based on the number of cigarettes regularly smoked by other family members at home and the cotinine levels in milk at baseline of the study, 5-7 days and 4 months after delivery. Milk samples were collected twice, 5-7 days and 4 months after delivery. The samples were stored at −20°C until assessment. The ELISA method (Cal biotech) was used to measure cotinine levels. Milk proteins including total protein and albumin were determined by spectrophotometry (Hitachi auto analyzer 7.7). Respondents were asked to report about food consumed over the previous 24 hours period (from midnight to midnight) using the validated and reliable 24-hour diet recall questionnaire (15). SPSS version 16.0 for Windows was used for data analysis. T-tests for continuous variables and Pearson $\chi 2$ test with the Fisher exact test for categorical variables were used to compare the two groups. $P \leq 0.05$ was considered as significant. All data were presented as mean ± SD.

4. Results

In demographic characteristics the number of employed mothers and maternal education levels in the non-exposed group was higher than that of the exposed group (Respectively P = 0.015 and P = 0.001) (Table 1). As expected, milk cotinine of mothers in the exposed group were significantly higher than those in the non-exposed group at baseline and 4 months after delivery. (P = 0.001 and P = 0.001, Respectively (Table 2). The levels of milk proteins in mothers in the non-exposed group were significantly higher than those in mothers in the exposed group at baseline (P = 0.001 for total protein and P = 0.004 for albumin) (Table 2). Results shows that the levels of total proteins in the exposed group (4 months after delivery) were also significantly higher than non-exposed group (P = 0.001). Dietary intake was not significantly different between the two groups at baseline and 4 months after delivery.

Table 1. Baseline and Socio-Demographic Characteristics of Studied Populations (n = 45) [a,b]

Variable	Exposed	Non-Exposed	P Value
Gender, Female	32 (62.7)	32 (62.7)	1
Infant age, day	4.74 ± 0.97	4.74 ± 1.03	1
Maternal age, y	26.07 ± 3.5	27.03 ± 3.5	0.717
Paternal age, y	31.05 ± 5.5	32.05 ± 4.6	0.144
Mothers' education, y	10.8 3.1	9.3 ± 3.1	0.015
Fathers' education, y	10.5 ± 3.6	9.4 ± 2.7	0.079
Parity number	1.6 ± 0.6	1.8 ± 0.6	0.239
Working mothers	0 (0)	11 (21.6)	< 0.001
Mothers' BMI, kg/m^2			0.425
19-24.9	7 (13.7)	10 (19.6)	
25-29.9	44 (86.3)	41 (80.4)	
Fahters' BMI, kg/m^2			0.525
19-24.9	36 (70.58)	33 (64.7)	
25-29.9	15 (29.42)	18 (35.3)	
Fathers' occupation			0.869
Skilled non-manual	4 (7.8)	2 (3.9)	
Skilled manual	1 (2)	1 (2)	
Semi-skilled	38 (74.5)	40 (78.4)	
unskilled	8 (15.7)	8 (15.7)	
Crowding index			0.651
<1 person	22 (43.1)	21 (41.2)	
1 person	25 (49)	28 (54.9)	
>1 person	4 (7.8)	2 (3.9)	
Insufficient family income	12 (23.5)	7 (13.7)	0.204

[a] Chi-square and Fisher exact tests were used for qualitative variables and the t test for quantitative variables that were not statistically significant at $P \leq 0.05$ level.
[b] Data are presented as No. (%) or Mean ± SD.

Table 2. Components of Milk in Exposed and Non-Exposed Groups (n = 45) [a]

Components of Milk	Exposed		Non-Exposed		P Value[b]
	Baseline[c]	4 Months[c]	Baseline[c]	4 Months [c]	
Total protein, g/dL	1.39 ± 0.49	1.07 ± 1.4	2.12 ± 0.91	4.2 ± 4.3	At baseline: 0.001, At 4 months: 0.001
Albomin, g/dL	0.69 ± 0.3	0.56 ± 0.33	0.88 ± 0.29	0.73 ± 0.83	At baseline: 0.004, At 4 months: 0.23
Cotinine, ng/dL	4.7 ± 1.2	4.1 ± 1.8	0.8 ± 1.9	1.3 ± 2	At baseline: 0.001, At 4 months: 0.001

[a] Data are presented as Mean ± SD.
[b] $P \leq 0.05$ is significant.
[c] Based on t- test.

5. Discussion

This study was the first in Iran to evaluate the effects of second hand smoke on breast milk proteins using cotinine as a biomarker of second hand smoke. Berlanga and colleagues in their study have reported up to 1027 ng /mL cotinine in breast milk (8). Also Orhon et al. in their study found mean cotinine among smoker mothers (13). In our study, the rate of cotinine was 6.43 ng/mL and 5.50 ng/mL 5-7 days and 4 months after delivery, respectively. On the other hand, the results of other studies showed increasing levels of cotinine in breast milk of smoker mothers. The limitation of this study was that it did not measure the exact amount of ventilation, illumination and duration of exposure to cigarette smoke. Although protein intakes, according to our dietary intake assessment, were similar in case and control groups, our data showed that protein levels were lower in the exposed than in the non-exposed groups both at initial and follow-up stages. Milnerowics et al. in their study showed that the protein content in milk of mothers exposed to cigarette smoke was less than their non-exposed counterparts (12). It seems cadmium contained in cigarette smoke induces the metallothionein production which is a protein-binding component that reduces the level of circulating proteins (12). Also This may be explained by an increase in lipoproteinas activity and lipoprotein metabolism induced by nicotine and its by-products in tobacco smokers (14). Previous studies have shown that second hand smoke in postpartum women is strongly associated with avoiding breast feeding (16, 17) possibly because of the effects of smoke on prolactin and dopamine secretion in the brain (15). Exposure to tobacco smoking affects breast milk proteins that are necessary for infants' development.

Authors' Contributions

Azar Shamsi: Study concept and design: Acquisition of data: Drafting of the manuscript: Study supervision: Azam Baheiraei: Study concept and design: Study supervision: Shahnaz Khaghani: Study concept and design Afshin Mohseni far: Study concept and design. Analysis and interpretation of data: Anooshirvan Kazemnejad: Statistical analysis.

Financial Disclosure

This study was funded and supported by Tehran University of Medical Sciences (TUMS).

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