

INCREASED CORD-BLOOD EOSINOPHIL COUNTS AND PRENATAL EXPOSURE TO TOBACCO SMOKE: A CONCERN FOR DEVELOPMENT OF ATOPY EARLY IN CHILDHOOD

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ABSTRACT

Objective: In adults several reports indicate that eosinophil counts and serum IgE levels increase in tandem with the number of cigarettes smoked. Several conflicting results have also been reported regarding prenatal cigarette smoke exposure and its effects on fetal immunoglobulin levels. Thus we surveyed cord-blood cotinine, IgE concentrations and eosinophil counts in 142 neonates in relation to parental smoking during pregnancy.

Methods: This study was performed at Marmara University Hospital between July 1995 and May 1996. Pregnant women were selected at random. Cord-blood was collected at delivery and maternal urine samples were collected within the first hour after delivery. Complete blood count and differential counts were measured in the cord-blood samples within the first hour after delivery. Serum was separated from the cord-

blood within 2 hours of collection and the specimens were stored at -30°C until assay, for cotinine and IgE.

Results: Maternal urinary cotinine/creatinine ratio (UCCR) was used as a biologic marker of passive smoke exposure. Maternal UCCR and cord-blood cotinine levels were significantly correlated, and both were highest in the active smokers and their infants. Cord-blood eosinophil counts rose in tandem with maternal UCCR. Cord-blood IgE levels were correlated with maternal UCCR only for nonatopic mothers. No relationship was found between cord-blood cotinine, IgE levels, and eosinophil counts.

Conclusion: We conclude that, maternal smoking, whether active and/or passive, affects cord-blood eosinophil counts in women without atopy. The implication of these findings in the development of atopy later in life have yet to be identified.

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INTRODUCTION

The relationship between parental smoking, neonatal cord-blood IgE levels, and atopic disease remains controversial. In adults, smoking increases both eosinophil counts (1) and serum IgE levels, especially in men (2). Investigations of prenatal passive smoking and cord-blood IgE levels have shown maternal smoking to be related to higher cord-blood IgE levels, especially in babies whose parents had no history of allergies (3). However, this finding has not been replicated in other studies (4-6). Thus the relationship between maternal smoking during pregnancy and cotinine, IgE concentrations, and eosinophil counts in cord-blood from 142 neonates were examined.

MATERIALS AND METHODS

This study was performed at Marmara University Hospital between July 1995 and May 1996. Pregnant women were selected at random, told of the study goals, and asked whether they would participate. After they gave informed consent, they completed standardized questionnaires concerning parental demographics, history of allergies and smoking habits. All women agreed to participate in the study, after they understood the fact that we do not inject the baby. None were taking antiallergy or antiasthmatic drugs. Women who smoked one cigarette or more per day during at least one trimester were classified as active smokers. Women who did not smoke but were exposed in their homes and/or work to at least one cigarette per day during pregnancy were classified as passive smokers. Nonsmokers were those who neither smoked nor were exposed to smoke in their homes and/or work during pregnancy. Information from the questionnaire was supplemented by tests of maternal UCCR and cord-blood cotinine status. Cord-blood was collected at delivery and maternal urine samples were collected within the first hour after delivery. Complete blood count and differential counts were measured in the cord-blood samples with a Coulter Plus within the first hour after delivery. Total numbers of

eosinophils per 100 cells on cord-blood smears were counted by an investigator who was blinded to the other results of the study. Serum was separated from the cord-blood within 2 hours of collection and the specimens were stored at -30°C until assay, for cotinine and IgE. Cotinine levels were determined in cord-blood and urine by liquid-phase radioimmunoassay (Double Antibody Nicotine Metabolite, Diagnostic Products Corporation, Los Angeles, CA). Cord-blood IgE levels were measured by Phadelbas IgE Prist^R 60, a sandwich technique immunoassay (Pharmacia AB, Uppsala, Sweden) and the results were expressed in IU/ml.

Maternal urinary creatinine was determined with a Spinreact kit (Pharmacia AB, Uppsala, Sweden) in which creatinine reacts with sodium hydroxide and picric acid to produce a colored complex, the rate of formation of which is measured spectrophotometrically. Urinary cotinine was expressed in nanograms per milligram of creatinine (UCCR). All tests were run in duplicates.

Data were analyzed by using "SPSS for windows" statistical software. Differences in parental characteristics were assessed with analysis of variance (ANOVA) used for comparing means and the chi-square test for comparing proportions. The Fisher exact test was used to detect associations between the infants' anthropometric characteristics and their parental exposure to smoking. Associations between IgE concentrations, eosinophil counts, cord-blood cotinine levels, and maternal UCCR were assessed with ANOVA or the Kruskal Wallis test. Relationships between variables such as cord-blood cotinine levels and UCCR were evaluated with the Pearson product moment correlation or the Spearman rank correlation tests. A p value of less than 0.05 was considered statistically significant.

RESULTS

Of the 142 neonates studied, 77 were male and 65 female (M:F ration 1.18:1). The mean gestational age of the infants was 39.3±1.18 weeks (range 37-42.5). Five (3.5%) were born prematurely, 134 (94.4%) were born at term, and

3 (2.11%) were born postmaturely. No correlations were found, between maternal smoking status and gestational age, height, weight, head or mid arm circumference, or triceps skinfold thickness (TSFT) (Table I). Maternal smoking status also was not associated with the infant's sex, presence of malformations, APGAR score, or type of delivery.

Of the 142 mothers, 61 were nonsmokers, 45 were passive smokers, and 36 were active smokers. The average age of the mothers was 26±5 years and that of the fathers was 29±6 years respectively. About 60% of the parents had completed only elementary school; 20% graduated high school; 20% had a college degree. In terms of occupation, only women who were civil servants had a significant higher rate of active smoking. Smoking habits were not correlated with other sociodemographic characteristics or with a history of atopy.

As expected, UCCR levels were higher in active smokers than in passive smokers, and lower still

in nonsmokers (Fig. 1). Cord-blood cotinine levels and maternal UCCR were significantly correlated ($r=84.54$, $p=0.0001$). Maternal UCCR correlated significantly with the daily number of cigarettes to which the mother was exposed ($\chi^2=29.5$, $p=0.0006$), but cord-blood cotinine did not (Table II).

Cord-blood eosinophil counts were significantly higher in infants born to active and passive smokers than in those born to nonsmokers ($\chi^2=26.9$, $p=0.00001$) (Fig. 1). Cord-blood eosinophil counts but not cord-blood cotinine levels increased in tandem with the maternal UCCR (Spearman $r=17.45$, $p=0.038$).

Cord-blood IgE levels were not related to maternal smoking status or the number of cigarettes to which the mother was exposed. Cord-blood IgE levels were correlated with maternal UCCR only for nonatopic mothers (Pearson $r=19.69$, $p=0.02$). No relationship was found between cord-blood cotinine and cord-blood IgE levels.

Table I: Neonatal Characteristics and Maternal Smoking Habits

Infant characteristics	Maternal Smoking Habits				p Value
	Nonsmoker	Passive Smoker	Active Smoker	Total	
Gestational age, wk	39.26±1.1	39.68±1.2	39.04±1.2	39.3±1.2	$p>0.05^{(1)}$
Height, cm	50.02±1.7	50.68±2.6	49.67±2.2	50.1±2.2	$p>0.05^{(1)}$
Weight, gr	3328.8±387	3499.5±534	3308.2±473	3377.7±464	$p>0.05^{(2)}$
Head circumference, cm	34.64±1.2	35.3±1.5	34.78±1.5	34.9±1.4	$p>0.05^{(1)}$
Mid-arm circumference, cm	10.66±0.91	10.76±1.02	10.67±0.97	10.7±0.95	$p>0.05^{(2)}$
TSFT*, mm	10.09±1.1	10.29±1.4	10.16±0.2	10.17±1.2	$p>0.05^{(2)}$
Units are means±standard deviations.					
(1) Kruskal Wallis test					
(2) Anova					
*Triceps skin fold thickness					

Table II: Cotinine Levels vs Daily Number of Cigarettes Smoked by Mother or Other Family Members

	Daily Number of Cigarettes Smoked by Mother or Other Family Members					p
	0	1-10	11-20	21-30	31-40	
Maternal UCCR, ng/mg creatinine	140.5±79	240.1±209	291.5±158	600.1±1402	1369.7±2773	$p=0.0006^{(1)}$
Cord-blood cotinine level, ng/ml	0.4±0.2	0.3±0.2	0.4±0.3	4.6±14.9	23±51.2	$p>0.05^{(1)}$
Units are means±standard deviations, UCCR, urinary cotinine:creatinine ratio.						
(1) Kruskal Wallis test						

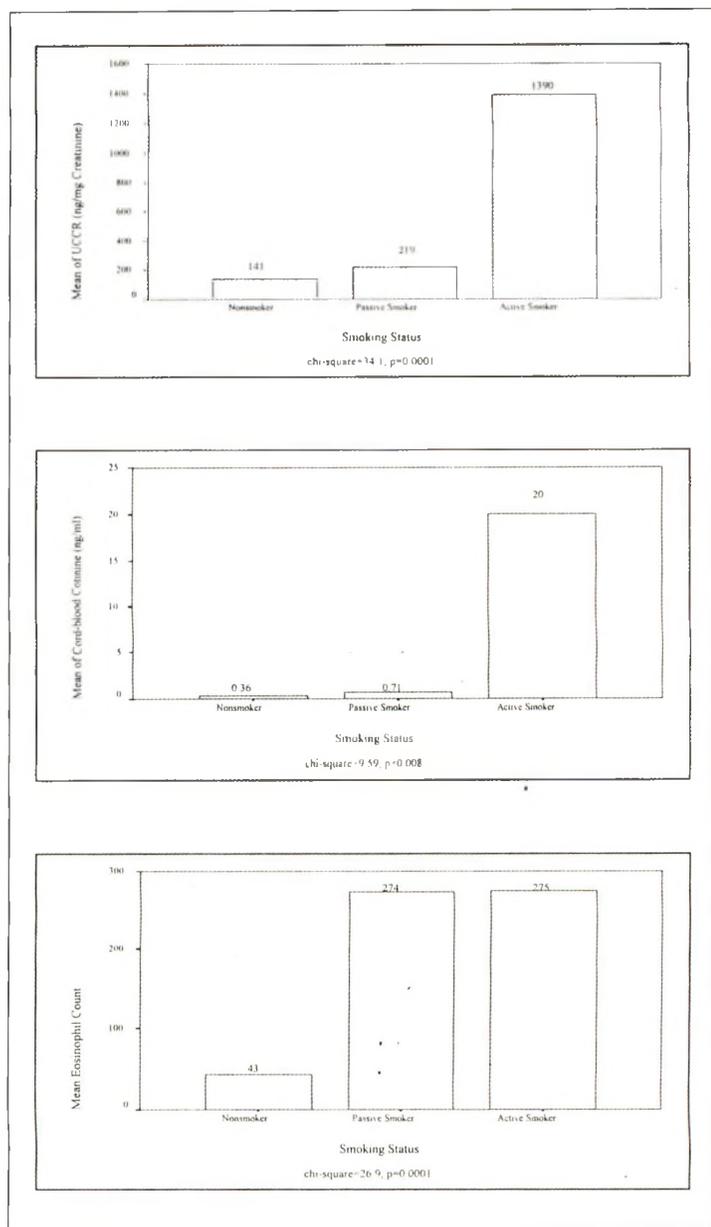


Fig. 1 : Maternal smoking status vs. maternal urinary cotinine:creatinine ratio (UCCR) (top), cord-blood cotinine (middle), and cord-blood eosinophilia (bottom)

DISCUSSION

We found that cord-blood eosinophil counts were higher in infants born to women who either smoked or lived with someone who smoked than in infants born to nonsmokers. Cigarette smoke and its components are readily transferred to the fetus throughout pregnancy (7-9). Several biochemical markers of tobacco smoke exposure, e.g. cotinine, nicotine, carbon monoxide, and thiocyanate, have been used to distinguish smokers from nonsmokers

(10). Cotinine and nicotine are both specific to tobacco, and are more reliable than carbon monoxide or thiocyanate for identifying smokers (11). Cotinine, which has a longer half-life than nicotine, is the most accurate marker, whether measured in saliva, urine or blood samples (11). Our results confirmed that both cord-blood cotinine and maternal UCCR can be used to assess prenatal exposure to cigarette. Although UCCR correlated with the number of cigarettes to which the prospective mothers were exposed, cord-blood cotinine levels did not.

In adults, several reports indicate that eosinophil counts and serum IgE levels increase in tandem with the number of cigarettes smoked (1,6); in one study, the absolute number of eosinophils were 47% higher in smokers than in nonsmokers (12). With regard to cord-blood, number of neutrophils was found to be significantly lower in-cord-blood of smoking mothers compared to nonsmoking mothers (13). In another study, in 129 neonates with family history of atopic diseases, sensitivity of using cord-blood IgE levels and eosinophil counts as a predictor of allergy ranged from 25% to 79% (14). With regard to infants, eosinophilia in the peripheral blood of apparently healthy 3 month old infants associated with subsequent atopic disease, positive skin prick test reaction, and elevated serum IgE levels (15). In a Norwegian cohort study, wheezing infants had significantly higher levels of serum eosinophil cationic protein compared with control infants (16). Pohunek et al studied eosinophils in bronchial biopsy specimens and suggested that increased numbers of eosinophils are risk factors for persistent asthma (17). Our study demonstrates the significant increase in cord-blood eosinophil counts with maternal passive and/or active smoking. So, it may be postulated that exposure to cigarette smoke in fetal life might be responsible for diseases in the future.

Several investigators have reported conflicting results regarding prenatal cigarette smoke exposure and its effects on fetal immunoglobulin levels (3-5, 18-20). In a 6-year prospective study, Martinez et al. found a direct correlation between persistent wheezing and IgE levels at 9 months of age but not between persistent wheezing and IgE levels in cord-blood (21). Magnusson et al found a significant association between maternal smoking and cord-blood IgE levels that was more pronounced for families with no prenatal history of allergies(3). In the present study, we found that cord-blood IgE levels were to be unrelated to exposure to tobacco smoke as documented by maternal UCCR, cord-blood cotinine and familial reports of smoking. IgE was correlated with maternal UCCR only for women with no history of atopy, a finding that leads us to postulate that hereditary aspects of atopy might overshadow the effect of tobacco smoke on neonatal IgE.

With regard to eosinophils, we found that cord-blood eosinophil counts, but not cord-blood cotinine, correlated with UCCR. Although cotinine is a reliable biological marker for tobacco-smoke exposure, it has some limitations, chief among them being that the results are accurate only for exposures during the previous 16 hours (10, 11). The lack of a marker for long-term tobacco smoke exposure, makes the interpretation of the results difficult. In our study, women were not expected to be exposed to tobacco smoke after the beginning of labour; given that labor can last at least 16 hours, cord-blood cotinine may not have been the ideal marker for this study.

Our finding of increased cord-blood eosinophils in the active and passive smokers even in the absence of correlation with cord-blood cotinine levels may be significant. Ronchetti et al. found that passive exposure to smoke among 9 year-old children both increased their serum IgE levels and led to a higher frequency of eosinophilia than in children whose parents did not smoke (22). In another study of healthy school children, the prevalence of high IgE and eosinophilia was higher in children whose parents smoked than in those whose parents were nonsmokers (23). These findings lead us to speculate that, cord-blood eosinophil count may be a more sensitive and longer-lasting indicator

of exposure to tobacco smoke than is cord-blood cotinine.

In conclusion, we found that exposure to tobacco smoke, during pregnancy whether active or passive, affects cord-blood eosinophil counts and perhaps IgE levels. The implications of these findings in the subsequent development of atopy have yet to be identified. Prospective studies following the same infants are under consideration.

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