

# Hematological Parameters and Cobalamin Status in Infants Born to Smoking Mothers

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## Key Words

Smoking mothers, newborn infants · Newborns, smoking mothers · Hematological parameters, smoking · Vitamin status, smoking · Homocysteine · Methylmalonic acid

## Abstract

Hematological parameters, serum cobalamin and folate levels, and the concentrations of the functional markers plasma methylmalonic acid and total homocysteine were determined in 173 newborns and 46 infants at 6 weeks to see whether maternal smoking influences the hematological parameters and the vitamin status of the newborn. At birth, there was a strong inverse correlation between the number of cigarettes smoked per day during pregnancy and red blood cell count ( $r = -0.56$ ,  $p = 0.001$ ) and hemoglobin level ( $r = -0.52$ ,  $p = 0.003$ ) in the newborns. Neonates born to smoking mothers had lower red blood cell counts and lower hemoglobin and serum cobalamin levels as compared with infants born to nonsmoking mothers. At 6 weeks, maternal smoking significantly predicted the methylmalonic acid and total homocysteine levels, suggesting an influence from smoking on the cobalamin function in these infants.

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## Introduction

Cigarette smoking during pregnancy is associated with an increased risk of complications, infant mortality, and disease [1–5]. Prenatal smoke exposure has growth-retarding effects [6–9] and may induce direct genetic damage in the fetus [10, 11].

The exact mechanism of fetal injury induced by maternal smoking is largely unknown. It has been linked with fetal hypoxia due to formation of carboxyhemoglobin and nicotine-induced vasoconstriction [12–14]. In adults, hypoxia is associated with increased levels of erythropoietin and the resulting erythrocytosis [15]. A higher level of erythropoietin has also been found in cord blood from newborns of smoking than in that from newborns of non-smoking mothers [16].

In adults, smoking is associated with decreased levels of cobalamin and folate [17–20] and elevated levels of total homocysteine (tHcy) [21]. Both low folate and cobalamin levels and high tHcy concentrations have been associated with an adverse pregnancy outcome [22–24]. A recent epidemiological study concluded that a regular use of multivitamin/mineral supplements might reduce the risk of fetal death associated with maternal smoking [25]. Thus, a deranged vitamin function may play a role

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in the negative effects associated with prenatal smoke exposure.

We have earlier reported [26] that the cobalamin status during the neonatal period is strongly associated with the maternal cobalamin status. Our results suggest that the prevalence of an impaired cobalamin status within the neonatal period may be underestimated. In infancy, nutritional cobalamin deficiency leads to dysfunction of the central nervous system, resulting in severe clinical symptoms and neurologic deterioration [27–30].

The aim of this study was to investigate whether maternal smoking during pregnancy influences the hematological parameters and the vitamin status of the newborn.

## Subjects and Methods

### Subjects

The participants were recruited among consecutive births, both vaginal and cesarean, of healthy, nonpremature (>35 weeks) newborns, registered at the Department of Obstetrics and Gynecology at Haukeland University Hospital in Bergen, Norway. Recruitment was done at random dates during the period from March 1996 to April 1997. All inpatients were asked to participate in the study, and only 2 mothers refused. Out of 186 births, 169 mothers and 173 newborns, including four sets of twins, were enrolled in the study. Pregnancies complicated by diabetes or Rh incompatibility and infants born to mothers on regular drug treatment were excluded, i.e., 13 newborns. All participants were invited for a second investigation after 6 weeks, and 43 mothers with a total of 46 infants chose to do so. The protocol was approved by the Regional Medical Ethics Committee, and written informed consent was obtained from all mothers.

### Data Collection

The gestational age was based on information of the last menstrual date, early ultrasound determination, and a pediatric examination of the newborn on day 1. Data on diet, smoking habits, and intake of vitamin supplements during pregnancy were obtained through an interview with the mother at the time of birth. Information on current and former pregnancies was obtained by interview at the time of birth and was checked against the data in the Medical Birth Registry of Norway.

Based on self-reported smoking habits, the participants were categorized into nonsmokers, which included never-smokers and ex-smokers (women who had stopped smoking during pregnancy), and current daily smokers. The reported mean number of cigarettes smoked per day was recorded. The intake of vitamin supplements was categorized as daily, sporadic, or no use. Parity was categorized as para 0 (no former children), para 1 (1 former child), or para 2+ (2 or more former children).

### Blood Sampling and Storage

Antecubital venous blood samples from the mother and the infant were collected on day 4 (96–108 h) after birth and after 6 weeks. The samples used for tHcy determination were placed in ice water, and plasma was separated within 2 h. Plasma and serum samples were stored at  $-20^{\circ}\text{C}$  until analysis.

### Biochemical Analyses

The hematological parameters were analyzed immediately, according to laboratory routine, using an H\*1 analyzer (Bayer, Tarrytown, N.Y., USA). Plasma methylmalonic acid (MMA) was assayed using a gas chromatography-mass spectrometry method based on ethyl chloroformate derivatization [31]. Plasma tHcy was determined by a liquid chromatography-tandem mass spectrometry method [32]. Serum and whole-blood folate were determined by a *Lactobacillus casei* microbiological assay [33] and serum cobalamin by a *Lactobacillus leichmannii* microbiological assay [34, 35]. Both folate and cobalamin assays were adapted to a microtiter plate format and carried out by a robotic workstation (Microlab AT plus 2; Hamilton Bonaduz, Bonaduz, Switzerland).

### Statistics

The results are presented as mean values  $\pm$  1 SD, median, and interquartile range or intertertile range. Daily smokers were divided into two groups based on the average number of cigarettes (7.4) consumed among smoking mothers. Such categorization created groups of similar size and allowed for dose-response estimates according to smoking. Comparisons between groups were performed by one-way analysis of variance and Student's *t* tests. Because the data were not normally distributed, correlations were assessed by Spearman correlation coefficients. Logistic regression was used to assess the influence of maternal smoking habits on the hematological parameters in the newborns. The outcome variables were low red blood cell count (lower tertile) or low hemoglobin level (lower tertile). Multiple linear regression was used to assess the simultaneous relation between maternal smoking habits and parity as predictors of serum cobalamin, plasma MMA, and plasma tHcy in the infant at birth. Two-sided *p* values  $<0.05$  were considered statistically significant. The SPSS statistical package (version 10) was used for all statistical analyses.

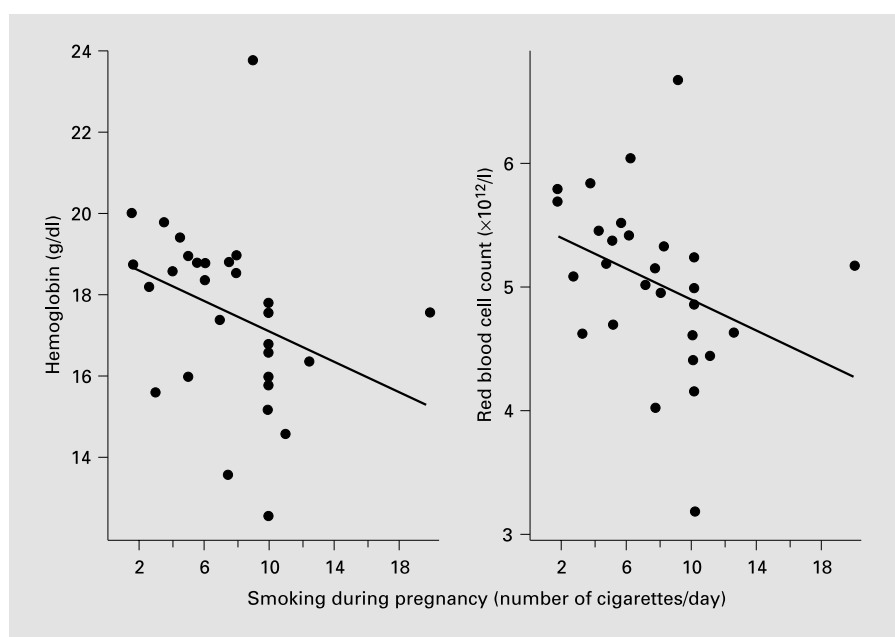
## Results

### Characteristics of the Study Population

The demographic characteristics of the study population according to maternal smoking habits during pregnancy are listed in table 1. The mean birth weight of the newborns was 305 g lower in the smoking group ( $p = 0.04$ ). The outcomes of the other parameters under investigation, gestational age, maternal age, parity, and vitamin supplement intake, were not significantly different between smokers and nonsmokers. The number of cigarettes smoked per day ranged from 2 to 20, with a mean of 7.4 (SD 3.9).

### Hematological Parameters

Both red blood cell count ( $r = -0.56$ ,  $p = 0.001$ ) and hemoglobin level ( $r = -0.52$ ,  $p = 0.003$ ) in the newborns were inversely related to the number of cigarettes smoked per day during pregnancy (fig. 1). Infants born to mothers smoking more than the average of 7 cigarettes/day had significantly lower red blood cell counts and hemoglobin



**Fig. 1.** Hemoglobin levels and red blood cell counts in newborns at birth in relation to the average number of cigarettes the mothers smoked per day during pregnancy.

**Table 1.** Sample characteristics of newborns and mothers at birth in relation to maternal smoking habits during pregnancy

	Nonsmokers	Smokers
<i>Newborns</i>		
Number	143 (included three sets of twins)	30 (included one set of twins)
Gestational age in weeks, median (interquartile range)	40 (39–41)	39.5 (38.8–40)
Birth weight in grams, median (interquartile range) <sup>a</sup>	3,750 (3,400–4,000)	3,445 (3,000–3,668)
Small for gestational age (%)	9 (6)	4 (13)
<i>Mothers</i>		
Number	140	29
Age in years, median (interquartile range)	29 (26–34)	29 (22–32)
Parity (%)		
Para 0	50 (35)	12 (40)
Para 1	56 (39)	9 (30)
Para 2+	37 (26)	9 (30)
Intake of vitamin supplements		
Sporadic or no use (%)	116 (83)	22 (76)
Daily (%)	24 (17)	7 (24)

<sup>a</sup>  $p = 0.04$ .

levels as compared with children born to nonsmoking mothers. The same trend, however, no longer significant, was seen at 6 weeks (table 2). There were no significant differences in mean corpuscular volume (table 2), leukocyte count, or platelet count (data not shown) according to smoking habits.

Using logistic regression, we assessed determinants of a low red blood cell count (lower tertile  $<5.08 \times 10^{12}/l$ ) and a low hemoglobin level (lower tertile  $<17.5$  g/dl) in the newborns at birth. Gestational age, birth weight, sex, and maternal factors, like parity, age, vitamin supplementation, and smoking habits, were included in the model as

**Table 2.** Hematological parameters in infants at birth and after 6 weeks in relation to maternal smoking habits during pregnancy (mean  $\pm$  SD)

Parameters	Nonsmokers	Smokers		p <sup>a</sup>	p <sup>b</sup>
		1–7 cigarettes/day	>7 cigarettes/day		
Number					
Birth	143	14	16		
6 weeks	38	2	6		
Red blood cells, $\times 10^{12}/l$					
Birth	5.33 $\pm$ 0.59	5.37 $\pm$ 0.41	4.79 $\pm$ 0.74	0.002	0.001
6 weeks	4.02 $\pm$ 0.50	4.18	3.67 $\pm$ 0.43	0.23	0.11
Hemoglobin, g/dl					
Birth	18.4 $\pm$ 2.0	18.4 $\pm$ 1.3	16.9 $\pm$ 2.6	0.02	0.008
6 weeks	12.6 $\pm$ 1.6	13.7	11.6 $\pm$ 1.7	0.24	0.17
Mean corpuscular volume, fl					
Birth	106 $\pm$ 5	107 $\pm$ 6	107 $\pm$ 4	0.40	0.33
6 weeks	93 $\pm$ 4	90	95 $\pm$ 4	0.31	0.32

<sup>a</sup> One-way analysis of variance.  
<sup>b</sup> Unpaired t test (nonsmokers vs. smokers >7 cigarettes/day).

independent variables. Smoking >7 cigarettes/day during pregnancy was the only significant predictor of a low red blood cell count or a low hemoglobin level (table 3). Additional adjustment for maternal levels of cobalamin and folate did essentially not change the odd ratios (data not shown). We found no significant correlation between newborn red blood cell count or hemoglobin level and serum cobalamin levels in either smoking group at birth.

#### *Vitamins and Metabolites*

The concentrations of vitamins and metabolites in infants at birth and after 6 weeks in relation to maternal smoking habits are presented in table 4. At birth, infants born to mothers smoking >7 cigarettes/day had significantly lower serum cobalamin levels as compared with the nonsmoking group. Serum and whole-blood folate levels and the metabolic markers, tHcy and MMA, were not significantly different between smokers and nonsmokers (table 4). At 6 weeks, the mean cobalamin level was reduced by approximately 40% in both smokers and nonsmokers, while the mean tHcy and MMA levels were increased in both groups. The increase in tHcy and MMA tended to be more prominent in infants born to mothers smoking >7 cigarettes/day, although the difference between the groups did not reach statistical significance (table 4).

The infant cobalamin status was the outcome measure in multiple linear regression models, with maternal fac-

**Table 3.** Odds ratio (OR) and 95% confidence interval (95% CI; in parentheses) for low red blood cell count or low hemoglobin level in newborns at birth according to maternal smoking habits during pregnancy

Smoking category <sup>a</sup>	n	OR (95% CI)	
		red blood cell count <5.08 $\times 10^{12}/l$ (lower tertile; tertile 1)	hemoglobin level <17.5 g/dl (lower tertile; tertile 1)
Nonsmokers	143	1	1
1–7 cigarettes/day	14	0.8 (0.2–3.1)	0.5 (0.1–2.0)
>7 cigarettes/day	16	5.6 (1.7–18.1)	3.8 (1.2–11.8)
p trend		0.004	0.01

<sup>a</sup> Adjusted for gestational age, sex, birth weight, maternal age, vitamin intake, and parity.

tors as independent determinants. The analysis demonstrated that maternal smoking was associated with significantly higher MMA and tHcy levels at 6 weeks (table 5).

## **Discussion**

In the present paper, we have demonstrated that neonates born to smoking mothers have lower red blood cell counts and hemoglobin levels at birth as compared with

**Table 4.** Vitamins and metabolites in infants at birth and after 6 weeks in relation to maternal smoking habits during pregnancy (mean ± SD)

Parameters	Non-smokers	Smokers		p <sup>a</sup>	p <sup>b</sup>
		1–7 cigarettes/day	>7 cigarettes/day		
Number					
Birth	143	14	16		
6 weeks	38	2	6		
Serum cobalamin, pmol/l					
Birth	378 ± 207	401 ± 184	287 ± 65	0.19	<0.001
6 weeks	232 ± 84	283	183 ± 74	0.25	0.18
Serum folate, nmol/l					
Birth	28.8 ± 10.9	26.4 ± 10.9	34.3 ± 15.1	0.12	0.18
6 weeks	26.5 ± 16.1	28.6	38.6 ± 29.0	0.33	0.14
Whole-blood folate, nmol/l					
Birth	580 ± 198	606 ± 256	580 ± 172	0.90	0.99
6 weeks	262 ± 85	486	322 ± 213	0.03	0.52
Plasma MMA, µmol/l					
Birth	0.36 ± 0.21	0.46 ± 0.60	0.30 ± 0.10	0.21	0.24
6 weeks	1.08 ± 1.19	1.25	3.11 ± 3.31	0.02	0.20
Plasma tHcy, µmol/l					
Birth	5.70 ± 1.82	5.87 ± 1.90	6.26 ± 2.07	0.50	0.25
6 weeks	7.76 ± 1.49	4.83	10.12 ± 5.03	0.01	0.30

<sup>a</sup> One-way analysis of variance.  
<sup>b</sup> Unpaired t test (nonsmokers vs. smokers >7 cigarettes/day).

**Table 5.** Maternal factors as determinants of serum cobalamin, plasma MMA, and tHcy levels in infants at birth (n = 173) and at 6 weeks (n = 46)

	Infant serum cobalamin				Infant plasma MMA				Infant plasma tHcy			
	birth		6 weeks		birth		6 weeks		birth		6 weeks	
	B	p	B	p	B	p	B	p	B	p	B	p
Smoking habits <sup>a</sup>	-41	0.09	-23	0.19	0.01	0.78	0.87	0.02	0.34	0.14	1.44	0.02

Multiple linear regression; the model contains gestational age, birth weight, sex, maternal age, vitamin intake, and parity in addition to the parameter listed in the table.

<sup>a</sup> Nonsmokers, 1–7 cigarettes/day, >7 cigarettes/day.

infants born to nonsmoking mothers. They also have lower serum cobalamin levels. Similar trends were observed at 6 weeks of age.

#### *Hematological Changes*

We observed an inverse correlation between number of cigarettes smoked per day during pregnancy and red

blood cell counts and hemoglobin levels in the newborns (fig. 1). Notably, the hematological parameters were measured in blood sampled 4 days after birth. Our data are in accordance with results of a study showing lower reticulocyte counts in cord blood in neonates of smoking mothers [36], but are in variance with those of several studies demonstrating either higher [16, 37] or equal [38, 39] hemato-

crit or hemoglobin concentrations in infants of smoking as compared with nonsmoking mothers. The data reported in the literature are thus inconsistent and are mostly based on measurements performed in cord blood [16, 36, 37] or in venous blood drawn within the 1st day after birth [38, 39]. The higher levels of hematocrit or hemoglobin have been related to increased levels of erythropoietin due to tissue hypoxia in neonates born to smoking mothers [16, 40].

The present study does not include measurements of erythropoietin levels or reticulocyte counts. This is a weakness, since it makes it difficult to assess the neonatal erythropoiesis. However, it is conceivable that discrepancies related to hematological parameters in newborns of smoking mothers may be partly related to the time of blood sampling, since pronounced alterations in hematocrit and blood viscosity are known to occur during the first hours of postnatal life [41].

#### *Cobalamin Status*

We have earlier reported that the cobalamin status during the neonatal period is strongly associated with the maternal cobalamin status [26]. In the present paper, these observations are extended by showing that the cobalamin status both at birth and after 6 weeks (tables 4, 5) is influenced by smoking during pregnancy. At 6 weeks, a pronounced increase in MMA and a decrease in cobalamin were detected in infants born to women smoking >7 cigarettes/day. Smoking could also be identified by multiple regression as a significant, strong predictor of an impaired cobalamin status (table 5). However, the observations at 6 weeks are based on small number of subjects, and the results should be confirmed in larger studies.

The negative effect of smoking during pregnancy on the cobalamin status of the newborns reported here is in accordance with published data. Smoking has been associated with reduced levels of cobalamin in adults [17, 42, 43], in pregnant women [18, 19], and in neonates born to smoking mothers [19]. Smokers tend to have a lower nutrient intake than nonsmokers [44]. Low levels of cobalamin in smokers have also been attributed to cyanide detoxification and increased urinary excretion of the vitamin [17].

Our observation of no association between cobalamin status and hematological parameters suggests that mechanisms different from an impaired cobalamin status may be responsible for the hematological changes in infants born to smoking mothers. Cigarette smoke contains an abundant number of toxic compounds that may interfere with various biological processes [10, 12]. However, the

observation that multivitamin/mineral supplementation reduces the risk of fetal death, particularly among heavy smokers [25], indicates a possible role of micronutrients in smoking-related fetotoxicity.

#### *Strength and Limitation of the Study Design*

Blood was drawn from a large vein of 173 infants on day 4 after birth. We thereby avoided the variability in hematocrit and low molecular weight compounds like vitamins and metabolites during the early postnatal period [41, 45, 46].

Although all participants were invited back after 6 weeks, we were only able to reinvestigate 46 infants. Among these, only 8 were born to smoking mothers. The low number of participants at 6 weeks results in limited statistical power. However, reductions in red blood cell counts, hemoglobin levels (table 2), and serum cobalamin concentrations (table 4) were observed in infants born to smokers at 6 weeks which adds credence to similar smoking effects observed at birth.

#### *Concluding Remarks*

Maternal smoking during pregnancy is associated with an increased risk of infant disease and mortality [2, 47]. The observed reductions in red blood cell counts and hemoglobin levels and also the reduced cobalamin status in infants born to mothers smoking >7 cigarettes/day may be related to some of these effects. The results should be confirmed in larger studies.

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