Concentrations of Ascorbic Acid in the Plasma of Pregnant Smokers and Nonsmokers and their Newborns

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Abstract: A cross-sectional study was carried out to determine the differences in vitamin C status of Brazilian pregnant women smokers and nonsmokers and their respective newborn babies, and to assess the prevalence of hypovitaminosis C among these two groups of women. The study involved 127 pregnant women, 40 pregnant smokers and 87 pregnant nonsmokers, admitted to a maternity hospital in São Paulo, Brazil. Data concerning the pregnant women's socioeconomic, demographic, obstetric, and nutritional characteristics were collected, as well as data concerning the newborns' anthropometry and Apgar scores. A strongly significant correlation ($p < 0.001$) was found between the concentrations of ascorbic acid (AA) in both pregnant smokers ($r = 0.77$) and pregnant nonsmokers ($r = 0.61$) and their respective umbilical cords. The mean umbilical AA concentration was significantly higher than the mean AA concentration in pregnant women (92.05 ± 41.13 vs. 33.39 ± 18.25 µmol/L, $p < 0.001$). It was observed that the mean AA was significantly lower for the newborns ($p = 0.03$) and pregnant women ($p = 0.02$) from the smoking group. Forty percent (40%) of the smokers and 27% of the nonsmokers presented hypovitaminosis C.

We suggest an increase in the consumption of fruits and vegetables by pregnant women, especially the smokers.

Key words: ascorbic acid, vitamin C, antioxidant, smoker, pregnant women, newborn babies

Introduction

An increase in reactive types of oxygen and of potential oxidation is an avoidable toxicological consequence, since it is inherent to all vital functions of aerobic organisms [1, 2]. At birth, the newborn is brought into an environment which is hyperoxic compared to *in utero* conditions and there occurs the greatest development of biochemical systems that may be decisive for the newborn's adaptation to oxygen [3]. These biochemical systems include antioxidant defense mechanisms that have the capacity to protect

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newborns against oxidative stress [1, 4]. The systems that maintain equilibrium and antioxidant defense use enzymatic and non-enzymatic strategies, of which ascorbic acid (AA) is considered the most important non-enzymatic water-soluble antioxidant [5]. AA is transported from the maternal circulatory system to the fetus through the placenta, where the fetal blood concentration of this vitamin is about twice that of the maternal blood concentration [6, 7]. The habit of smoking is directly related to concentrations of AA in the blood. A reduction of 30% to 40% in plasmatic AA levels has been confirmed in individual smokers, when compared with nonsmokers [8]. This reduction is attributed mainly to two factors: an inadequate diet with low intake of antioxidants, especially vitamin C [9, 10] and increased rate of metabolism of this vitamin in smokers [11, 12]. Cigarette smoking is a source of oxidant stress in pregnant women, and probably in the fetus [9]. Various studies suggest that the low concentrations of AA during pregnancy have been associated with an increased risk of contracting infections, premature rupture of membranes [13, 14], pre-eclampsia [15], low birth weight, premature birth, and intra-uterine growth retardation [16].

Therefore, in the present study we decided to: (1) determine the differences in vitamin C status of Brazilian pregnant women, smokers and nonsmokers, and their respective newborn babies; (2) assess the prevalence of hypovitaminosis C among pregnant smokers and nonsmokers.

Methodology

This cross-sectional study included 40 pregnant smokers, 87 pregnant nonsmokers and their respective newborns. The pregnant women were selected at the Obstetric Center of the University of São Paulo University Hospital. Any woman who indicated that she had smoked during her pregnancy, regardless of the number of cigarettes smoked daily, was considered a smoker. Low-risk pregnant women were included in the study, while pregnant women with histories of infectious or metabolic diseases, hypertension, preeclampsia/eclampsia, and those who had given birth to twins, or those with gestational ages of < 37 or ≥ 42 weeks were excluded. Socioeconomic demographic, obstetric, and nutritional information was obtained through interviews with the pregnant women. Gestational weight gain was calculated by subtracting the weight of the pregnant women upon admission to the Obstetric Center from the weight at beginning of the pregnancy. The questionnaire was administered depending on the state of the woman and efforts were make to find a propitious time before or after delivery. Anthropometric data for the newborns and other birth related data were obtained through birth certificate records at the Obstetric Center. Babies were measured for length in their cradles six hours after birth. Blood samples to determine AA concentrations were collected from the umbilical cord within 10 minutes of placenta delivery, and samples of maternal blood were obtained in the pre-delivery room. All of the samples were collected in Vacutainer® (Becton Dickinson) tubes which contain the anticoagulant sodium heparin. After centrifuging, the plasma was stored in a freezer (–86°C), for a period of no longer than four days, while awaiting analysis. The AA concentration was determined by the Department of Clinical and Toxicological Analysis and Pharmaceutical Sciences at the University of São Paulo using high-performance liquid chromatography (HPLC) with ultraviolet (UV) detection at a wavelength of 254 nm on plasma previously deproteinized with metaphosphoric acid [17]. Criteria based on the Nutrition Canada interpretive guidelines were used to classify risk of clinical scurvy in pregnant smokers and nonsmokers and their newborns [18]. Women with plasma AA concentrations of 14.1 μmol/L (0.2 mg/dl) or less were considered to be at high risk for clinical vitamin C deficiency (severe hypovitaminosis C), while those with plasma concentrations of 22.7 μmol/L (0.4 mg/dL) or less were considered to be at marginal risk (marginal hypovitaminosis C). The program Statistical Package for the Social Sciences (SPSS) version 10.0 was used for the statistical analysis. The correlation between the levels of blood AA in the pregnant women and in their umbilical cords, comparing the groups of pregnant smokers and nonsmokers, was obtained using Pearson's correlation coefficient (r). In comparing the two groups of pregnant women and their newborns, the differences between the means were evaluated by the Student's *t*-test. The mean values are described with their respective standard deviations (± SD). A probability value of < 0.05 was considered significant.

The study was approved by the Research Ethics Commission at the University of São Paulo (USP) School of Public Health and the Research Ethics Committee at the USP University Hospital. After an explanation of the study, both the researcher and pregnant women signed an informed consent agreement in duplicate.

Results and discussion

In this study, data from 127 pregnant women, 40 of which were smokers and 87 nonsmokers, and their respective newborns were analyzed. Tables I and II show the population distribution studied with some characteristics of the pregnant women and their newborns.

Table I: Characteristics of 40 smokers pregnant women and the 87 nonsmokers pregnant women. São Paulo, 2002

Characteristics	Pregnant women (S) n	Pregnant women (S) %	Pregnant women (NS) n	Pregnant women (NS) %
Age (years)				
< 20	12	30.0	16	18.4
20–34	23	57.5	67	77.0
≥ 35	5	12.5	4	4.6
Per capita income (BMW)[a]				
< 1	25	62.5	38	43.7
1|— 2	13	32.5	30	34.5
2|— 5	2	5.0	15	17.3
5|— 10	–	0.0	3	3.4
> 10	–	0.0	1	1.1
Education (years)				
1–4	11	27.5	10	11.4
5–8	15	37.5	39	44.9
≥ 9	14	35.0	38	43.7
Number of pregnancies #				
Primiparous	10	25.0	42	48.3
Multiparous 2–4	28	70.0	40	46.0
Multiparous ≥ 5	2	5.0	5	5.7
Parity				
0	12	30	48	55.2
1–2	21	52.5	30	34.5
≥ 3	7	17.5	9	10.3
Type of delivery				
Vaginal	24	60.0	41	47.1
Cesarean section	13	32.5	27	31.1
Forceps	3	7.5	19	21.8
BMI[b]				
< 20	1	2.5	1	1.1
20–25	5	12.5	11	12.7
25–30	18	45.0	51	59.8
> 30	16	40.0	23	26.4

S = Smokers, NS = Non-smokers, # Significant difference (p = 0.04)
n = number of observations
[a] 1 Brazilian Minimum Wage = 67 US dollars
[b] Body mass index at interview (kg/m^2)

All the participants were insured by the National Health Service under the Unified Health System (SUS), which provides assistance to low-income families.The majority of pregnant women earn low wages and only 16.5% earn more than 2 Brazilian minimum wages (1 Brazilian minimum wage = US$ 67.00). This fact prevents this class of people from ingesting a balanced diet that would provide all the nutrients required for proper gestation and development of the newborn. Most of the pregnant women, 77%, were between 20 and 34 years of age, which is considered a low obstetric risk range.

Out of the pregnant smokers group, 32 (80%) stated that they had smoked for all three trimesters of their pregnancy.

Table II: Characteristics of the 40 newborn babies of smoking women and of the 87 newborn babies of nonsmoking women. São Paulo, 2002

Characteristics	Newborn babies (S) n	Newborn babies (S) %	Newborn babies (NS) n	Newborn babies (NS) %
Gestational age (weeks)[a]				
37–38	18	45.0	33	37.9
39–40	21	52.5	40	46.0
41	1	2.5	14	16.1
Weight at birth (g)#1				
< 2500	1	2.5	–	0.0
2500 |— 4000	36	90.0	84	95.6
≥ 4000	3	7.5	3	3.4
Height at birth (cm)#2				
<47	15	37.5	19	21.8
47 |— 50	20	50.0	47	54.0
≥ 50	5	12.5	21	24.2
Apgar (1-minute)[b]				
2–5	1	2.5	5	5.8
6–7	–	0.0	11	12.6
8–10	39	97.5	71	81.6
Apgar (5-minute)				
2–5	–	0.0	1	1.1
6–7	–	0.0	–	0.0
8–10	40	100.0	86	98.9

S = Smokers, NS = Non-smokers, # Significant difference,
#1 (p = 0.02), #2 (p = 0.01)
n = number of observations
[a] Gestational age evaluated by the Capurro method [26]
[b] Apgar Scores [27]

Table III shows the behavior of the pregnant smokers during the period and the number of cigarettes smoked daily.

Table IV shows that the mean plasmatic concentrations of AA were higher in the newborns than in their mothers. A significant difference was also observed in the mean AA concentrations between the pregnant smokers and nonsmokers (p = 0.02), and between the newborns of the two groups (p = 0.03). Other studies have also shown higher concentrations of AA in newborns, compared to their mothers, indicating selective retention by the fetus [19] in addition to the AA that is actively transported through the placenta in newborns taken to term [3, 7, 20]. Infants delivered in a group of nonsmoking mothers had significantly higher mean birth weights and heights than infants in a smoker group (p < 0.02 and p < 0.01), respectively. Other authors have observed similar results [21, 22]. The group of pregnant smokers included in our study presented a 21.5% lower concentration of AA than the nonsmoking group. Similar studies carried out in the United States and Spain found reductions of 24% [23] and 12% [10] respectively, comparing smoking and nonsmoking pregnant women.

Table III: Number of cigarettes smoked by the pregnant women in the three different trimesters of pregnancy. Sao Paulo, 2002

	Trimesters						Total	
Cigarettes/day	1st, 2nd and 3rd		2nd and 3rd		1st and 3 rd			
	n	%	n	%	n	%	n	%
1–5	10	25.0	1	2.5	1	2.5	12	30.0
6–10	9	22.5	2	5.0	1	2.5	12	30.0
11–15	7	17.5	2	5.0	–	0.0	9	22.5
16–20	4	10.0	–	0.0	–	0.0	4	10.0
>20	2	5.0	1	2.5	–	0.0	3	7.5
Total	32	80.0	6	15.0	2	5.0	40	100.0

n = number of observations

Table IV: Mean plasma concentrations of ascorbic acid (AA) in smokers and nonsmokers women and respective newborn babies. São Paulo, 2002

AA (µmol/L)	Pregnant women (S)		Pregnant women (NS)	
	mean (± SD)	n (%)	mean (± SD)	n (%)
Plasma Pregnant women	28.13a (15.45)	40 (31.5)	35.82b (18.99)	87 (68.5)
AA (µmol/L)	Newborn babies (S)		Newborn babies (NS)	
	mean (±SD)	n (%)	mean (±SD)	n (%)
Plasma Newborn babies	80.45 c (39.16	40 (31.5)	97.38 d (41.13)	87 (68.5)

S = Smokers, NS = Non-smokers, n = number of observations
a ≠ b ($p = 0.02$)
c ≠ d ($p = 0.03$)

The results of this study showed that 40% of the pregnant smokers, 27% of the pregnant nonsmokers, 2.5% of the newborns with smoking mothers, and 1.1% of the newborns with nonsmoking mothers had concentrations that fall within the range of marginal hypovitaminosis C. Previous studies found a similar prevalence of marginal hypovitaminosis C for a smoking group (43.9%) [10], and a lower prevalence for a nonsmoking group (12.7%) [24] of pregnant women. The lower prevalence of marginal hypovitaminosis C in this study is perhaps due to the fact that the population studied has higher standards of living since it was selected from middle class Canada, where the population has probably greater access to information on healthy diets and possesses the financial resources to pay for it. Pfeffer [14] emphasizes that an increased prevalence of vitamin C deficiency may indicate a high rate of subclinical infections, which could be the cause of an increased vitamin C uptake by the peripheral tissues. According to Ladipo [25] the concentration of AA falls progressively during pregnancy, partly due to hemodilution, which may result in an increase of 35–40% in the blood volume, but also due to the transfer of AA to the fetus. We see, therefore, that the prevalence of marginal hypovitaminosis C in groups of smoking pregnant women at the same stage of pregnancy, even in populations with higher incomes, does not differ from that observed in the present study, which presents the results of a markedly less affluent group. The reduction in plasmatic AA and the consequent increase in the prevalence of hypovitaminosis C observed in the smokers may be due to malabsorption or an increase in metabolism, based on studies that measured the concentrations of AA in urine concomitantly with the administration of known doses of the vitamin [23]. Figures 1 and 2 show that the levels of AA in the newborns of smokers and nonsmokers, respectively, increase as the levels of AA concentrations in their mothers increase, which confirms a strong positive correlation in both groups ($r = 0.77$, $p < 0.001$; Fig. 1) and ($r = 0.61$, $p < 0.001$; Fig. 2). Significant correlations in previous studies were also observed [3, 19, 24].

Conclusion

A high prevalence of hypovitaminosis C was detected among the pregnant women: 40% of the pregnant smokers and 27% of the pregnant nonsmokers presented hypovitaminosis C.

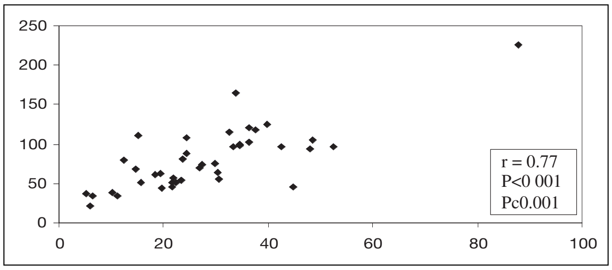

Figure 1: Correlation between ascorbic acid (AA) concentrations in smokers pregnant women and respective newborn babies. São Paulo, 2002.

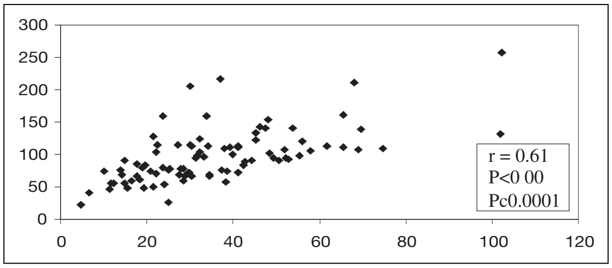

Figure 2: Correlation between ascorbic acid (AA) concentrations in nonsmokers pregnant women and respective newborn babies. São Paulo, 2000.

Strong positive correlations were found in the plasmatic concentrations of AA between smoking and nonsmoking pregnant women and their respective newborns.

The mean AA concentration in the umbilical cord was significantly higher when compared with the mean AA concentration in the pregnant women, suggesting that some selective retention mechanism is at work to ensure that the fetus will be protected against oxidative stress during part of its adaptation to oxygen, despite the deficient concentrations in its mother.

The data of this study permit us to conclude that the high prevalence of detected marginal hypovitaminosis C is most likely related to the low dietary supply of vitamin C and an elevated metabolic consumption of AA in the pregnant smokers' group, which are reflected in lower concentrations in their respective newborns.

We suggest an increase in the consumption of fruits and vegetables by pregnant women, especially the smokers, which should be stimulated through strategies of education with the participation of health professionals.

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