Letter to the Editor

The level of ascorbic acid in the amniotic fluid of smoking and non-smoking women in labour

Željko Trošelj[1], Martin Gredičak[6], Gordana Planinić Radoš[2], Ratimir Klepac[3], Rajko Fureš[7], Zlatko Hrgović[7], Dražan Butorac[4], Dubravko Habek[5]

Clinical Hospital Center Zagreb, Female Health Clinic[1], Department of Obstetrics and Gynecology Clinical Hospital Merkur[2], Department of Biology, School of Medicine, University of Zagreb[3], Department of Gynecology and Obstetrics Clinical Hospital Centre „Sestre milosrdnice"[4], Department of Gynecology and Obstetrics Clinical Hospital „Sveti Duh"[5], Zagreb, General Hospital Zabok, Department of Obstetrics and Gynecology, Zabok[6], Faculty of Medicine Osijek, Josip Juraj Strossmayer University of Osijek, Osijek[7], Croatia

Dear Editor,

We would like to report our recent findings that point to remarkable differences in the levels of soluble proteins and ascorbic acid measured in amniotic fluid, which can be associated with the smoking habits of the mother.

Embryonic and foetal growth in mammals depends on the flow of nutrients and oxygen from the mother. All soluble organic and inorganic matter is transferred from the mother to the foetus through the placental barrier, which includes compounds from various harmful sources. One such source is tobacco smoke, a mixture of over 5,000 different inorganic and organic compounds, many of which are carcinogenic (1-3). Children of smoking mothers commonly experience deleterious health effects.

Reactive oxygen species (ROS) are an important source of damage to biologically important macromolecules like DNA, proteins, lipids and polysaccharides. They can have either exogenous or endogenous origin, the latter including intracellular sources, e.g. mitochondria (4). Each organism counteracts harmful ROS-mediated effects through various antioxidant molecules, among which are vitamins C and E, albumin, haemoglobin, uric acid, and enzymes such as catalase or superoxide dismutase (5). Considerable amounts of antioxidants originate from one's diet, if it is rich in fruit and vegetables.

A large influx of ROS leads to oxidative stress, which creates a microenvironment prone to pathogenic processes (6). Every cigarette one smokes induces a certain level of oxidative stress (7). The aforementioned vitamin C (or ascorbic acid) has been confirmed as an effective neutralizer of ROS, as it releases hydrogen atoms and transforms into dehydroascorbic acid (4). Smokers exhibit as many as three-fold lower vitamin C levels in plasma than non-smokers (8).

Correspondence to: Prof Dubravko Habek, MD, MSc, PhD, University Department of Obstetrics and Gynecology, University Hospital „Sveti Duh", Sveti Duh 64 Street, Croatian Catholic University, 10000 Zagreb, Croatia, Fax: 00385 1 3745534, Tel: 00385 91 3712112, e-mail: *dubravko.habek@os.t-com.hr*

In a pilot study we conducted within our own institutions, we examined the level of soluble proteins and ascorbic acid in the amniotic fluid of twenty smoking and non-smoking women in labour. Nine of the mothers were non-smokers, 4 occasional smokers, and 7 permanent smokers. Occasional smokers smoked 4-10 cigarettes per day and stopped smoking once they had become aware of their pregnancies. The permanent smokers had smoked up to 15 cigarettes a day until they became aware of their pregnancies and then reduced that number to 2-4 cigarettes per day.

Applying standard methodology (9, 10), the amniotic fluid samples were prepared for protein analysis and colorimetric measurement of vitamin C levels. The obtained results (see Table 1) were expressed in µg ascorbic acid per mL of amniotic fluid.

The amniotic fluids of non-smoking and occasionally smoking mothers contained similar quantities of proteins. At the same time, the level of proteins in the amniotic fluid of permanent smokers was twice as low.

The differences in the quantities of ascorbic acid were even greater. The highest quantity was recorded in non-smokers, while the other two groups exhibited 6 to 7 times lower values.

The ascorbic acid values we observed in the smoking mothers deserve particular attention, as a deficiency of ascorbic acid may have a detrimental effect on foetal growth and development and pose a potential risk. It should also be mentioned that the concentration of ascorbic acid in amniotic fluid may vary considerably in long-lasting childbirths due to additional stress.

The decrease of ascorbic acid in amniotic fluid was associated with the decrease in protein quantity, which was possibly influenced by diminished protein synthesis in the foetal metabolism. It is also quite possible that low levels of vitamin C may lead to the impaired physiological functioning of a developing organism (11, 12).

Table 1 *Concentration of soluble proteins and ascorbic acid in the amniotic fluid taken from non-smoking, occasionally smoking, and permanently smoking women in labour*

Group	Amniotic fluid	
	Proteins mg mL^{-1}	Ascorbic acid µg mL^{-1}
Non-smokers	2.04±0.01	574.20±59.22
Occasional smokers	2.03±0.02	83.21±8.89^{s}
Permanent smokers	1.09±0.04^{s}*	91.55±9.89^{s}

s=p<0.001 with regard to non-smoking
**=p<0.001 with regard to occasional smokers*

Our preliminary results suggest that macromolecular analysis of amniotic fluid could point to the harmful effect that tobacco smoke has on foetal growth.

REFERENCES

1. Pryor WA, Stone K. Oxidants in cigarette smoke. Radicals, hydrogen peroxide, peroxynitrate, and peroxynitrite. Ann NY Acad Sci 1993;686:12-27. doi: 10.1111/j.1749-6632.1993.tb39148.x
2. St Charles FK, McAughey J, Shepperd CJ. Methodologies for the quantitative estimation of toxicant dose to cigarette smokers using physical, chemical and bioanalytical data. Inhal Toxicol 2013;25:383-97. doi: 10.3109/08958378.2013.794177
3. Remmer H. Passively inhaled tobacco smoke: a challenge to toxicology and preventive medicine. Arch Toxicol 1987;61:89-104. doi: 10.1007/BF00661366
4. Krinsky NI. Mechanism of action of biological antioxidants. Proc Soc Exp Biol Med 1992;200:248-54. doi: 10.3181/00379727-200-43429
5. Dröge W. Free radicals in the physiological control of cell function. Physiol Rev 2002;82:47-95. doi: 10.1152/physrev.00018.2001
6. Dalle-Donne I, Rossi R, Colombo R, Giustarini D, Milzani A. Biomarkers of oxidative damage in human disease. Clin Chem 2006;52:601-23. doi: 10.1373/clinchem.2005.061408
7. Frei B, England L, Ames BN. Ascorbate is an outstanding antioxidant in human blood plasma. Proc Natl Acad Sci USA 1989;86:6377-81. PMID: 2762330
8. Lykkesfeldt J, Loft S, Nielsen JB, Poulsen HE. Ascorbic acid and dehydroascorbic acid as biomarkers of oxidative stress caused by smoking. Am J Clin Nutr 1997;65:959-63. PMID: 9094879
9. Jagota SK, Dani HM. A new colorimetric technique for the estimation of vitamin C using Folin phenol reagent. Anal Biochem 1982;127:178-82. doi: 10.1016/0003-2697(82)90162-2
10. Lowry OH, Rosebrough NJ, Farr AL, Randal RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951;193:265-75. PMID: 14907713
11. Barrett BM, Sowell A, Gunter E, Wang M. Potential role of ascorbic acid and beta-carotene in the prevention of preterm rupture of fetal membranes. Int J Vitam Nutr Res 1994;64:192-7. PMID: 7814234
12. Pressman EK, Thornburg LL, Glantz JC, Earhart A, Wall PD, Ashraf M, Pryhuber GS, Woods JR Jr. Inflammatory cytokines and antioxidants in midtrimester amniotic fluid: correlation with pregnancy outcome. Am J Obstet Gynecol 2011;204:155.e1-7. doi: 10.1016/j.ajog.2010.08.064