Abstract. – OBJECTIVES: In utero fetal exposure to tobacco smoke has been found to be associated with adverse pregnancy outcome and increased maternal and fetal risks. The aim of this study was to compare umbilical cord blood S100B levels of infants of active smoker, passive smoker and non-smoker mothers.

SUBJECTS AND METHODS: A total of 82 women, 26 habitual smokers, 27 passive smokers and 29 controls, who were admitted for repeat elective cesarean delivery with uncomplicated term gestations were included in the study. The age, gravidity, parity and gestational week at delivery were recorded on admission for the delivery. Ultrasonographic evaluation was routinely done on admission and birth weights of the newborns were measured immediately upon delivery. Umbilical cord blood was collected following delivery of the infants and serum S100B levels were analyzed at the end of the study period. The groups were compared according to S100B levels.

RESULTS: No significant difference was found between the three groups regarding age, gravidity, gestational week at delivery or birth weight of the infants (p > 0.05). Biparietal diameter of the fetuses of active smoker mothers were significantly smaller than passive smokers and controls (90.3 ± 1.8 vs 94.2 ± 2.8 and 93.8 ± 2.5, respectively). Mean S100B level in the umbilical cord blood of active smokers was lower than passive smokers and controls (768.9 ± 446.9 vs 1050.1 ± 383.2 and 1035.3 ± 405.2) (p = 0.024).

CONCLUSIONS: Fetuses of active smoker mothers had lower cord blood S100B levels, suggesting a possible injury of glial cells.

Key Words: S100B, Smoking, Environmental tobacco exposure, Cord blood.

Introduction

Maternal smoking is the single most widespread perinatal insult in the world1. In utero fetal exposure to tobacco smoke has been found to be associated with adverse pregnancy outcome and increased risks of maternal, fetal and infant morbidity and mortality across populations2,3. It was reported that in 2002, 5%-8% of preterm deliveries, 13%-19% of term low-birth-weight deliveries, and 5%-7% of preterm-related deaths were attributable to prenatal smoking in the United States4. The prevalence of smoking among pregnant women has been shown to vary across different countries, ranging from 9.9% in Japan to 30-35% in Spain. Despite the well known and detrimental effects, smoking remains prevalent among pregnant women5. Moreover, over the last two decades passive smoking, which is the exposure of a nonsmoker to harmful effects of smoke from a burning cigarette or smoke exhaled by a smoker, has gained attention as a public health problem6. There is a growing body of evidence regarding the negative effects of both active and passive smoking during pregnancy7,8.

S100B is an acidic calcium binding protein that is concentrated in the cytoplasm and can be actively released by astro- and oligodendrocytes of the nervous system and was also shown to be present in many biological fluids of the body9-11. It is involved in the regulation of many cellular processes, such as cell growth and differentiation, protection from oxidative damage, contraction, protein phosphorylation and secretion12. This protein exhibits cytokine-like activities at lower concentrations, acting as a growth and differentiation factor for neurons and astrocytes, while it could be neurotoxic and induce apoptosis at higher concentrations13-15. S100B is proven to be a useful marker of brain injury to adults, with an excellent negative predictive value12,16. Studies evaluating S100B concentrations in perinatology also showed that an elevated S100B concentration in biological fluids, such as cerebrospinal fluid, cord blood, peripheral blood, and urine is a marker of brain damage in fe-
Statistical Analysis

All statistical analysis was performed using IBM SPSS Statistics Software (19.0, SPSS Inc., Chicago, IL, USA). Mean ± standard deviation (SD) is presented for continuous variables. The independent samples t-test was used for continuous variables while the Mann-Whitney U test was applied for nonparametric variables. Chi-square test was used for comparison of categorical values. Pearson and Spearman correlation tests were used for evaluation of the correlation between variables. Statistical significance was assumed with a probability error at $p < 0.05$.

Results

A total of 82 pregnant women, 26 active smokers, 27 passive smokers and 29 non-smokers were involved in this study. Mean number of cigarettes smoked by active smokers was $5.8 ± 2.4$ per day. Demographic characteristics of the women in the three groups are shown in Table I. No significant difference was found between the three groups regarding age, gravidity or gestational week at delivery ($p > 0.05$) (Table I). Although no significant difference was found for birth weight of the infants in the three groups, infants of active smokers were smaller than infants of controls or passive smokers ($3159.2 ± 451.2$ vs $3348.9 ± 445.3$ and $3331.8 ± 355.3$, respectively). Biparietal diameter of the fetuses of active smoker mothers was significantly smaller than for passive smokers and controls ($90.3 ± 1.8$ vs $94.2 ± 2.8$ and $93.8 ± 2.5$, respectively).

Mean umbilical cord blood S100B levels of active smokers was lower than passive smokers and controls ($768.9 ± 446.9$ vs $1050.1 ± 383.2$ and $1035.3 ± 405.2$) ($p = 0.024$). Post-hoc tests revealed that the difference between controls and active smokers or between controls and passive smokers was not statistically significant ($p > 0.05$); however, the difference between active and passive smokers was statistically significant ($p = 0.045$). Umbilical cord S100B levels showed a weak but statistically significant negative correlation with the number of cigarettes smoked by active smokers per day ($r = -0.280, p = 0.011$).

Discussion

S100B is an acidic calcium binding protein primarily produced by glial cells and Schwann...
Table I. Demographic characteristics and umbilical cord S100 B levels of the patients.

<table>
<thead>
<tr>
<th></th>
<th>Controls (n=29)</th>
<th>Passive smokers (n=27)</th>
<th>Smokers (n=26)</th>
<th>p values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>29.7± 4.9</td>
<td>27.1± 4.1</td>
<td>28.3± 4.8</td>
<td>0.112</td>
</tr>
<tr>
<td>Gravidity</td>
<td>2.34± 0.72</td>
<td>2.59± 0.89</td>
<td>2.88± 1.2</td>
<td>0.118</td>
</tr>
<tr>
<td>Gestational age (weeks)</td>
<td>39.2± 0.4</td>
<td>39.3± 0.6</td>
<td>39.2± 0.5</td>
<td>0.766</td>
</tr>
<tr>
<td>BPD (mm)</td>
<td>93.8± 2.5</td>
<td>94.2± 2.8</td>
<td>90.3± 1.8</td>
<td>0.001*</td>
</tr>
<tr>
<td>Sex of the infant</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>17 (58.6%)</td>
<td>14 (51.9%)</td>
<td>18 (69.2%)</td>
<td>0.430</td>
</tr>
<tr>
<td>Female</td>
<td>12 (41.4%)</td>
<td>13 (48.1%)</td>
<td>8 (30.8%)</td>
<td></td>
</tr>
<tr>
<td>Birth weight (gr)</td>
<td>3348.9± 445.3</td>
<td>3331.8± 355.3</td>
<td>3159.2± 451.2</td>
<td>0.238</td>
</tr>
<tr>
<td>S100 B level (pg/mL)</td>
<td>1035.3± 405.2</td>
<td>1050.1± 383.2</td>
<td>768.9± 446.9</td>
<td>0.024*</td>
</tr>
</tbody>
</table>

BPD, biparietal diameter.

cells, which is released into the blood when the blood-brain barrier is disrupted\textsuperscript{11,37}. When overexpressed, it enhances neuroinflammation and neuronal apoptosis. Measurement of S100B protein offers an alternative for assessing cell damage in the nervous system\textsuperscript{11}. It has been defined as a useful marker of brain injury in adults. Studies have also shown that S100B is a predictive marker of hypoxic cerebral lesions in the fetus\textsuperscript{11,38}. Although S100B is extensively studied as a marker of injury in growth retarded or preterm fetuses, the relation between smoking and S100B concentration has not been studied yet.

Tobacco is one of the most heavily abused drugs in the world, with an estimated 21% of adults in the US identifying themselves as regular smokers\textsuperscript{21}. The smoke of tobacco contains over four thousand chemicals\textsuperscript{22}, many of which are pharmacologically active, mutagenic and carcinogenic\textsuperscript{23,24}.

Active smokers inhale the tobacco smoke produced at high temperatures, which is filtered through the smoker’s lungs. On the other hand, smoke produced at lower temperatures during the slow spontaneous combustion at the tip of the cigarette between puffs is inhaled by passive smokers\textsuperscript{23-25}. In either way, nicotine and carbon monoxide easily cross the placenta and accumulate in fetal tissues\textsuperscript{26}. There is evidence that the concentration of nicotine in fetal circulation is 15% higher than that found in maternal circulation and the concentration of nicotine in the amniotic fluid is 88% higher than in the maternal plasma\textsuperscript{27}. Nicotine can affect the fetus via vasoconstriction of the uteroplacental vasculature leading to underperfusion and a reduction in nutrient and oxygen flow to the fetus\textsuperscript{28}. Carbon monoxide also binds hemoglobin to form carboxyhemoglobin resulting in depletion of oxygen supplied to fetal tissue and hypoxia.

Nicotine effects in the cardiovascular system have been well documented in previous studies. Lindblad et al\textsuperscript{29} demonstrated that large doses of nicotine, as seen in heavy smokers, can change fetal heart rate and umbilical blood flow. Other studies\textsuperscript{13,30} also confirmed this relation, showing increased resistance in umbilical artery blood flow in smokers. In utero exposure to nicotine was also shown to affect the central nervous system and brain development. Previous experimental studies showed brain weight was significantly decreased by nicotine treatment in rats\textsuperscript{31}. Maternal smoking during pregnancy was also found to be associated with reduced growth of the fetal brain in humans\textsuperscript{31-34}. Lv et al\textsuperscript{31} demonstrated that fetal exposure to nicotine not only induced brain growth restriction associated with fetal hypoxia, but also affected the expression of nicotinic acetylcholine receptors, which are essential for brain development. Reduced size of the fetal head was also demonstrated by Köllen et al\textsuperscript{35}. The results of our study are in line with the previous studies demonstrating decreased biparietal diameter in the fetuses of active smokers. Chang et al\textsuperscript{36} compared brain metabolite levels of twenty-six children with prenatal nicotine exposure and 24 nicotine-unexposed children. The prenatal nicotine exposure group had lower concentrations of myoinositol and total creatine than controls. They concluded that this may be due to decreased glial content or altered glial development. Decreased levels of umbilical cord S100B levels may also be an indirect indication of nicotine induced glial cell damage as shown in this study. The lower levels of S100B detected in the active smoker group compared to passive smokers and controls may result from destructive effects of active smoking on the glial cells of the brain, which in turn decrease the number of these cells and even-
tually decrease the concentrations of S100B. This hypothesis was also supported by the finding of decreased biparietal diameter of active smokers in our study. However, we could not demonstrate an effect of passive smoking on S100B levels.

Data in the literature about the relation between birth weight and S100B concentrations is more clear. An inverse relationship between maternal smoking and birth weight and growth retardation has been shown in many previous studies. Studies measuring S100B concentrations in growth retarded fetuses documented different results. Boutsikou et al. reported no significant difference between serum S100B concentrations between growth retarded neonates and control groups, whereas Florio et al. demonstrated increased urinary S100B levels in growth retarded neonates with abnormal neurological outcome. The lack of difference could possibly be attributed to the brain sparing effect in the case of growth retardation, so the glial cells producing S100B are not affected by malnutrition. Also, no difference was found between S100B concentrations between small for gestational age and normal weight fetuses. In our study, infants of active and passive smoker mothers were appropriate for gestational age. Although infants of active smokers were smaller than controls, the difference was not statistically significant.

Conclusions

The present study constitutes the first using cord blood S100B levels to evaluate possible brain damage due to maternal smoking. Our results showed that fetuses of active smoker mothers had lower cord blood S100B levels. This suggests possible injury of glial cells caused by active smoking. However, this theory needs to be supported by larger studies.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References


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