Role of oxidative injury in affecting the foetal & placental weights on exposure to tobacco smoke

Huma Musarrat Khan,1 Muhammad Yunus Khan,2 Liaqat Ali Minhas3

Abstract
Objective: To investigate the role of oxidative injury in affecting foetal and placental weights in mice by exposing them to tobacco smoke with or without supplementation with antioxidants.

Methods: The randomized control trial of pregnant mice at day one of gestation was conducted at Anatomy Department CPSP Regional Center Islamabad, from March 2005 to October 2005. The mice were divided into three groups: Group C had controls, while the two other groups, groups S and SV were exposed to secondary tobacco smoke in a whole body exposure chamber with and without supplementation with vitamins respectively. At term, the animals were sacrificed and the placentae and foetuses were weighed. The average values were calculated. The means for each group were analysed and the foetal placental ratio was calculated. SPSS 17 was used for statistical analysis.

Results: There were 44 mice; 15(34%) each in S and SV groups, while Group C had 14(32%) mice who acted as the controls. The mean foetal weight in Group S was 0.65±0.52g which was significantly less (p<0.0001) than 1.48±0.19g in Group C. The mean foetal weight in Group SV was 0.97±0.65g which was not significantly different from S (p=0.124). The mean placental weight in Group S was 0.16±0.02g which was significantly less than 0.21±0.05g in Group C (p=0.014). In Group SV it was significantly more than Group S (p<0.0001). The ratio of mean foetal and mean placental weights in the groups C, S and SV were 7.05, 3.92 and 4.41 respectively.

Conclusion: Prenatal exposure to tobacco smoke decreased the mean foetal and placental weights and the foetal-placental ratio. This may partly be attributed to oxidative injury induced by free radicals in the tobacco smoke as it is prevented to some extent by simultaneous administration of antioxidants.

Introduction
Exposure to tobacco smoke during intrauterine life may lead to a variety of disturbances in development. Intrauterine growth retardation and low birth weight is one of the most commonly faced problems. Over 20% of the low birth weight incidence in the United States may be attributed to prenatal exposure to cigarette smoke.1 Low birth weight is an important predisposing risk factor to increased neonatal mortality2 and even if the infants survive the initial few months of their life, they face an increased risk of long-term health issues such as mental deficits, obesity, hypertension, cardiac diseases and diabetes.3,4

The relationship between tobacco exposure and foetal development has also been confirmed by epidemiological studies. Although the possible underlying pathological mechanisms have not been thoroughly investigated in humans, but animal studies suggest an impact of atmospheric contaminants such as tobacco smoke on placental structure, weight and function. Successfull pregnancy demands continuous interactions between the embryo and mother for which the normal structure and functioning of placenta is necessary. The placenta weight which reflects placental structure and function in turn has a nonlinear relation to the birth weight and is an important predictor of birth weight.6 Placental weight and its ratio to birth weight have recently been reported to predict chronic disease in later life.7 Previous studies have shown a decrease in placental weight,6 and multiple histological changes in the placentae exposed prenatally to tobacco smoke9 which may be responsible for compromised placental functioning, ultimately leading to low birth weight.

Tobacco smoke contains many noxious and cancer-causing chemicals,10 as well as unstable free radicals and reactive oxygen species which have the potential for causing oxidative damage.11 These are normally counteracted by antioxidants such as vitamin C and E,12 thereby neutralising the free radicals and their harmful effects. If free radicals are not neutralised by the body, oxidative injury ensues in which the free radicals adversely alter the cell biochemistry and trigger a number of diseases. Therefore, it is suggested that...
Supplementation of exogenous antioxidants can assist in overcoming this oxidative stress (OS) and preventing oxidative injury (OI). There is hence a requirement to focus on the role of dietary antioxidant supplements which might prove effective in preventing the free radicals associated oxidative damage to tobacco-exposed placenta. This in turn may help to improve the foetal birth weight and thus decrease perinatal mortality.

The current study was designed to investigate the role of OI in affecting the foetal and placental weights in pregnant mice by exposing them to tobacco smoke with or without supplementation with antioxidants.

**Material and Methods**

The randomized control trial of pregnant mice at day one of gestation was conducted at Anatomy Department CPSP Regional Center Islamabad, from March 2005 to October 2005. The Balb C strain mice were selected using tables of random sampling. Pregnancy was confirmed by observing the vaginal plug, the presence of which was considered as day 1 of gestation (1 day post coital [DPC]). The mice were housed in metal cages— not more than 6 per cage -- and food and water was provided to them ad libitum. They were divided into three groups: Group C had 14 controls; Group S and Group SV had 15 mice each.

Group S was exposed to passive cigarette smoke in a whole body exposure chamber. Five of the mice from this group were exposed to 4 cigarettes daily with a spacing of 30 minutes between each cigarette from 7 DPC to the date of sacrifice (Mild smoke). Ten mice from Group S were exposed to 12 cigarettes daily from 7 DPC onwards with a spacing of 15 minutes between each (Moderate smoke); Group SV was also subdivided into two subgroups and exposed to Mild (n=5) and Moderate smoke (n=10). The interval between two smoke exposures was based on pilot studies (30 minutes and 15 minutes in case of Mild and Moderate smoke exposure respectively) and was meant to ensure exposure to fresh air as is encountered in normal human smoking. The mice in Group SV were given intramuscular (IM) injections of sodium ascorbate (vitamin C) daily in the dose of 35mg/kg body weight in the morning before exposure to smoke. In addition, their diet was enriched with vitamin E supplements (400 international units in 20kg of food). This routine was carried out 5 days a week.

At 19 DPC, the animals were sacrificed and dissected. The abdomen was opened and the foetuses were separated from the placentae by cutting the umbilical cord. The placenta and the foetuses were dried by dabbing on a blotting paper, and weighed on an electronic balance of readability of up to 0.001g. The average weight of the foetuses and placenta for every case was calculated. SPSS 17 was used for statistical analysis. The mean and standard deviation (SD) for each group was analysed. The means were compared for statistical significance using Student’s t test at a confidence interval (CI) of 95 percent.

**Results**

There were 44 mice: 15(34%) each in S and SV groups, while Group C had 14(32%) mice who acted as the controls. The mean foetal weight in Group S was 0.65±0.52g which was significantly less (p<0.0001) than 1.48±0.19g in Group C. The mean foetal weight in Group SV was 0.97±0.65g which was not significantly different from S (p=0.124). The mean foetal weight in group foetuses exposed to Mild smoke was 1.09±0.18g, which was significantly less than Group C (p=0.001). Upon exposure to Moderate smoke the mean foetal weight further decreased to 0.49±0.50g. This was also significantly less (p=0.025) compared to the Mild smoke group. In Group SV, the mean foetal weight was significantly greater than Group S among the Mild as well as Moderate exposure to smoke (p=0.033, p=0.025 respectively) (Tables 1 and 2).

The mean placental weights in Group S was 0.16±0.02g, which was significantly less (p=0.014) than Group C in which the mean placental weight was 0.21±0.05g. However, this value in placenta of Group SV was significantly more than Group S (p<0.0001).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Foetal weight (g) Mean ± SD</th>
<th>Placental weight (g) Mean ± SD</th>
<th>Ratio of Mean Foetal &amp; Mean Placental weights</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Group n =14</td>
<td>1.48±0.19</td>
<td>0.21±0.05</td>
<td>7.05</td>
</tr>
<tr>
<td>Smoke Group n=15</td>
<td>0.65±0.52</td>
<td>0.16±0.02</td>
<td>3.92</td>
</tr>
<tr>
<td>Smoke plus Vitamin Group n=15</td>
<td>0.97±0.18</td>
<td>0.23±0.41</td>
<td>4.41</td>
</tr>
<tr>
<td>Mild Smoke group n =5</td>
<td>1.09±0.18</td>
<td>0.17±0.23</td>
<td>6.26</td>
</tr>
<tr>
<td>Moderate Smoke Group n =10</td>
<td>0.49±0.50</td>
<td>0.16±0.02</td>
<td>3.06</td>
</tr>
<tr>
<td>Moderate Smoke plus Vitamin Group n =5</td>
<td>1.37±0.16</td>
<td>0.24±0.02</td>
<td>5.71</td>
</tr>
<tr>
<td>Moderate Smoke plus Vitamin Group n =10</td>
<td>1.14±0.55</td>
<td>0.23±0.03</td>
<td>4.96</td>
</tr>
</tbody>
</table>
The mean placental weight in the group exposed to Mild smoke was 0.17±0.23 g. In the group exposed to Moderate smoke, the mean placental weight was 0.16±0.02 g, which was significantly less than Group C (p=0.031). However, there was no statistically significant difference in the placental weights in the Moderate compared to Mild Group S (p=0.401). Moreover, a significant increase in weight was seen in Group SV exposed to Mild smoke (p=0.025), and a highly significant increase was seen in Group SV exposed to Moderate smoke compared to their counterparts in Group S (p<0.0001). The foetal:placental ratio was calculated for the various groups and was seen to decrease as the exposure to smoke increased (Figure). This ratio in Group C was 7.05 which decreased to 3.92 in Group S and 4.41 in Group SV.

**Discussion**

Birth weight is considered a strong predictor of infant survival. In the present study, significantly lower mean foetal weight was observed in the mice exposed to smoke compared to the control group. This is in accordance with a number of animal and human studies. In some of these studies, the pregnant female rats when exposed to cigarette smoke led to low birth weights.16,17 Numerous human studies have also indicated the relationship of cigarette smoke with reduced foetal weight.18 This may be due to premature births,19 or decreased foetal growth.20,21 However, in this project, no pre-term delivery was observed, so it can be assumed that the reduced foetal weight was due to intrauterine growth retardation. This is once again in accordance with other studies that indicate reduced foetal growth rather than pre-term delivery to be responsible for increased perinatal mortality in babies exposed to tobacco smoke in utero.22

In the present study a decrease in the average foetal weight was observed as the exposure to smoke increased. However, simultaneous administration of antioxidants led to a significant improvement in mean foetal weight. However, this weight was still less than the mean foetal weights in the control group. Thus, the antioxidants only partially ameliorated the harmful effects of smoke.

The present study also showed a statistically significant decrease in the placental weight upon exposure to smoke which was significantly prevented in the group which was given antioxidants along with exposure to smoke. This can be compared with some studies which claim reduced neonatal weight at birth and an indistinct weight reduction of the placenta, thereby leading to an increase in placenta foetal ratio.5 Yet other studies claim no differences in maternal, placental and neonatal parameters between the exposed and non-exposed groups.23 In spite of these contradictory studies, sufficient evidence exists to confirm that smoke exposure of the placenta affects the placental metabolism which may be an important mediator of adverse effects induced by smoke exposure.24

**Table-2:** Statistical significance in foetal weights and placental weights among various groups.

<table>
<thead>
<tr>
<th>Statistical Significance in Foetal Weight</th>
<th>Statistical Significance in Placental Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Control and Smoke groups</td>
<td>p&lt;0.0001**</td>
</tr>
<tr>
<td>Between Smoke and Smoke plus Vitamin group</td>
<td>p=0.124</td>
</tr>
<tr>
<td>Between Mild Smoke group and Mild Smoke plus Vitamin group</td>
<td>p=0.033*</td>
</tr>
<tr>
<td>Between Moderate Smoke group and Moderate Smoke plus Vitamin group</td>
<td>p=0.014*</td>
</tr>
<tr>
<td>Between Mild Smoke group and Moderate Smoke group</td>
<td>p=0.025*</td>
</tr>
<tr>
<td>Between Control and Mild Smoke group</td>
<td>p=0.001**</td>
</tr>
<tr>
<td>Between Control and Moderate Smoke group</td>
<td>p=0.031**</td>
</tr>
</tbody>
</table>

Student “t” test used to compare statistical significance of means
Significant: p<0.05*, Highly Significant: p<0.001**

**Figure:** Mouse foetus (case C101b: Control group) seen attached to placenta. Cut section of placenta (block face) can also be seen.
In contrast to these findings related to smoke exposure, studies regarding the effect of first trimester maternal malnutrition on foetal and placenta weight claim an increase in placental weight, but not in birth weight. It has been suggested that the increase in placental weight may be a compensatory mechanism to maintain placental functioning which may be impaired as a result of reduction in maternal caloric intake.25 However, no such compensatory growth was observed in the present study, indicating that exposure to tobacco smoke caused some kind of injury to the placenta. This view is further strengthened by other studies which indicated histological changes in smoke-exposed placentae such as thickening of the maternal foetal barrier which can impair the normal functioning of the placenta.26

The foetal-placental ratio is an important indicator of foetal well-being. In the present study, a decrease in the foetal: placental ratio was observed upon exposure to smoke. However, as the Apgar score was not noted, the effect of this on the foetal well-being cannot be commented upon which can be considered a limitation of the present study. Previous studies showed that foetal: placental ratio of more than 10.00 is associated with significantly greater risk of low Apgar score (less than 6).27 This is evident by studies which showed that pre-eclampsia is accompanied by an increase in foetal: placental ratio along with decreased foetal and placental weights thereby compromising the foetal outcome.28 This indicates that a decrease in this ratio, as seen in the present study, may be a predictor of a good Apgar score and thus better foetal well-being.

Another limitation of the study is the small sample size, which was because of financial constraints and decreased availability of experimental animals. It is therefore recommended that further studies should be conducted in which these parameters may be related with Apgar score and foetal well-being. Moreover, the sample size may be increased in accordance with the formula recommended for calculating the sample size.

**Conclusion**

Prenatal exposure to tobacco smoke decreased the mean foetal and mean placental weight. This decrease may partly be due to oxidative injury induced by free radicals in the tobacco smoke as it is prevented to some extent by simultaneous administration of antioxidants.

**References**


