Original Article

# Effect of Prenatal Cigarette Smoke Exposure on Maternal Fetal Barrier and its Possible Prevention

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Background: Prenatal Exposure to cigarette smoke has deleterious effects on the placenta and the fetus, which may be due to toxic substances and free radicals present in smoke. The latter cause oxidative injury and therefore, the role of antioxidants need to be investigated as a possible preventive agent.

Objective: To determine, the effects of cigarette smoke on maternal fetal barrier and to observe the preventive role of antioxidants if any.

Materials and Methods: 51 female mice (Balb C strain) were mated and grouped: Groups C: control, S: exposed to smoke and SV: exposed to smoke and given antioxidants (vitamin C, E) and sacrificed at 19 dpc (days post coitus). 14 animals from C, 12 from S and 14 from SV had healthy pregnancies. Their placentae were studied microscopically. The thickness of the maternal fetal barrier and the distance between mononuclear trophoblast cells in the maternal sinusoids were measured.

Results: The thickness of maternal fetal barrier, and the distance between mononuclear trophoblast cells in the S group (2.21±1.35µm, 38.36±2.05µm) were significantly greater than in the Control (1.31±0.04µm, 33.48±1.00µm) with P=0.0001 and P=0.01 respectively. The thickness of the barrier in the SV (1.66±0.09µm) was significantly thinner than the S group (P=0.002).The distance between mononuclear trophoblast cells in the SV did not increase significantly (33.58±1.37µm: P=0.17) as compared to the Control.

Conclusion: Cigarette smoke causes thickening of the maternal fetal barrier which could be ameliorated significantly by antioxidants. Therefore, these effects may be partly due to oxidative injury produced by free radicals present in the smoke.

*Key Words:Cigarette smoke. Free radicals. Antioxidants*

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## Introduction

Cigarette smoking is an international problem and according to an estimate by WHO, if the present smoking patterns continue, smoking is expected to be responsible for death of 10 million people every year by 2020.[1] Cigarette smoke contains many toxic chemicals[2], and a high concentration of free radicals which may cause oxidative injury in the body.[3] It is associated with oxidative stress leading to an up regulation of antioxidant systems; which in turn help in reducing the oxidative load.[4] Cigarette smoke harms nearly all systems of the body reducing quality of life and life expectancy. As fetuses have immature systems and their antioxidant enzymatic processes are not fully developed, therefore if exposed to smoke (through the placenta) *in utero,* they form a vulnerable population for adverse effects.[5] Since placenta is a maternofetal organ which is needed for the exchange of gases, nutrients and waste products between the mother and fetus, any environmental insult that affects the placenta can lead to placental insufficiency, fetal growth retardation and even death.[6] Therefore prenatal exposure to cigarette smoke, along with other injurious effects, has also been linked with low birth weight and increased perinatal mortality.[7]

Studies regarding the effects of cigarette smoke on the morphology of human placenta have shown a number of histological changes linked with tobacco exposure. These include an increase in thickness of subtrophoblastic membrane[8] and hyperplasia of cytotrophoblast cells.[9] These observations relate with an impaired exchange of gases and nutrients between the mother and fetus, thereby increasing the risk of under nourishment of the fetus.[10] Similar studies on mice have also shown adverse effects of cigarette smoke on mouse placenta.[11]

The laboratory mouse belongs to the order Rodentia and is a good example of the placentalia. In mice, the

main placenta is chorioallontoic, which is made up of a single cotyledon which is discoid. Three regions can be distinctly identified in the placenta from without inwards.

1. **Giant cell layer:** They are homologous to the extravillous cytotrophoblast cells in the humans, and are responsible for accomplishing implantation.[10]
2. **Spongiotrophoblast layer:** These cells are homologous to the cytotrophoblast cell columns in the primates.[12]
3. **Labyrinthine Layer:** This layer constitutes the major site of maternal fetal exchange. The barrier between the maternal and fetal blood being hemochorial[6]. It is homologous to the chorionic villi in the primates. The trophoblast cells with their associated fetal vessels and maternal channels create this densely packed layer. The chorionic trophoblast cells differentiate to create a trilaminar epithelium.

- A layer of mononuclear trophoblast cells lying adjacent to the maternal vascular channel.
- An intermediate layer of syncytiotrophoblast cells.
- Another layer of syncytiotrophoblast cells which is in direct apposition to the endothelial cells of the fetal derived blood vessels and separated from them only by the basement membrane. [6]

Although experimental studies are available regarding the beneficial effects of antioxidants in neutralizing some of the harmful effects of tobacco smoke, however direct histological study of placenta and the extent of the preventive effects produced by antioxidant has not been carried out. Keeping this in mind an experimental study was planned using the laboratory mice. The objective of the study was to observe the effects of cigarette smoke on histology of mouse placenta especially the thickness of the maternal fetal barrier and to observe the role of antioxidants in preventing those changes.

# Materials and Methods

This randomized control trial was carried out at the Anatomy department CPSP Regional Center Islamabad, from February to December 2005. A total of 51 nulliparous mice (*Balb C* strain) were selected by random sampling.

The mice were housed in standard cages in a temperature and humidity controlled room on a twelve hour dark and light cycle. They were taken for breeding, and every four of these were placed in a male's cage. They were checked daily for the presence of vaginal plug, which was considered as day 1 of gestation (1 dpc: days post coital). The mice with vaginal plugs were weighed, and divided into three groups. Six mice were housed in a metal cage of shoe box size and food and water provided to them “ad libitum”.

1. Control group (Group C : 17 animals)
2. Smoke group (Group S: 17 animals):

This group was further divided into two subgroups and were exposed to passive cigarette smoke in a whole body exposure chamber as described in a previous study.[13]

- Exposed to mild smoke (n=6): They were exposed to 4 cigarettes daily from 7 dpc, with an interval of half hour in between.
- Exposed to moderate smoke (n=11): These were exposed to 12 cigarettes daily from 7 dpc onwards with an interval of 15 min in between.

3. Smoke plus Vitamin group (Group SV: 17 animals):

This group was further subdivided into two subgroups and exposed to smoke. They were injected vitamin C (sodium ascorbate) intramuscularly (35mg/Kg body weight)[14], and were provided with vitamin E supplemented diet (400 international units in 20 kg of food).

Exposed to mild smoke and given vitamins (n=5): They were exposed to 4 cigarettes daily from 7 dpc, with an interval of half hour in between and were given vitamin C and vitamin E supplements.

- Exposed to moderate smoke and given vitamins (n=12): These were exposed to 12 cigarettes daily from 7 dpc onwards with an interval of 15 min in between, and were given vitamin C and vitamin E supplements.

All the animals in the three groups were sacrificed on 19 dpc. The abdomen was opened and the uteri were examined. The animals with apparently healthy pregnancies in different groups were included in this study and are given below, whereas the remaining animals were used in another study.

1. Control group (n=14):
2. Smoke group (n=12):

- 5 animals were exposed to mild smoke
- 7 were exposed to moderate smoke.

3. Smoke plus vitamin group (n=14):

- 5 animals exposed to mild smoke
- 9 exposed to moderate smoke.

The uteri with attached placentae of these animals were stored in 10% formalin, and the tissues were processed for paraffin embedding. Five micrometer thick sections were cut and stained with haematoxylin and eosin stain, and PAS stain. The labyrinthine layer of the placenta was studied under the 100x oil immersion objective lens and following observations were made.

a. **Maternal fetal barrier**: The maternal sinusoids were identified as those vascular spaces which had large mononuclear trophoblast cells projecting into their lumen. The other vessels were regarded as fetal capillaries. The barrier between these two vessels was considered as the maternal fetal barrier. In the PAS stained slides, the barrier was measured at five different sites where it was relatively thin. The measurement was taken with the help of a previously calibrated ocular micrometer and average thickness was calculated.

b. **Distance between mononuclear cells**: In haematoxylin and eosin stained slides a maternal sinusoid containing two or more mononuclear trophoblast cells were identified. Photographs using a digital camera were taken from 3 such sites. These photographs were then transferred to a morphometric computer software named "ImageJ 1.33"[15] which is used for measuring distances of user defined selections. The distance from the nucleus of one mononuclear cell to the nucleus of the other cell was outlined by freehand tool and the distance calculated with the above mentioned software. Three such readings were taken, and the average distance between two mononuclear cells was calculated.

The means and standard error of the two variables in all the groups were calculated compared for significance using paired sample t test at a confidence limit of 95 percent.

# Results

The labyrinthine layer made up the major portion of the placental disc. It consisted of thin fetal capillaries lined by endothelial cells. These capillaries were surrounded by trophoblast cells which separated them from the maternal sinusoids. All the vessels were arranged in a more or less longitudinal fashion extending from the fetal surface towards the decidua. The labyrinthine trophoblast cells had a rounded nucleus, a clear nucleolus and basophilic cytoplasm.

a. **Maternal fetal barrier:** On examination under the oil immersion lens at 100x, the maternal fetal barrier could easily be identified.

b.

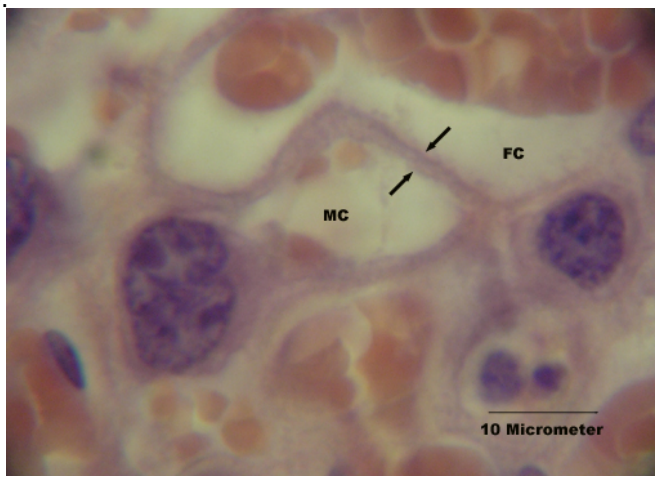


**Figure I: Section of labyrinthine layer of mouse placenta (case C8b) showing maternal fetal barrier (arrow) between fetal capillary (FC) and maternal sinusoid (MS). Haematoxylin and eosin stain. Photomicrograph. Bar, 10µm**

c. The mean maternal fetal barrier in the Smoke group (2.21±1.35µm) showed a statistically highly significant thickening with a P value of 0.0001 as compared to the 19 dpc placentae of Control group (1.31±0.04µm). This thickening was significant even on mild exposure to smoke (P<0.05). The mean maternal fetal barrier in the Smoke plus Vitamin group was 1.66±0.09µm which is significantly more than the 19 dpc placentae of Control group with a P value of 0.003, and significantly less than the Smoke group: P=0.002. The decrease of thickness in Smoke plus Vitamin group as compared to the smoke group was significant even on moderate exposure to smoke: P< 0.05(Table I and Figure 1, 2).

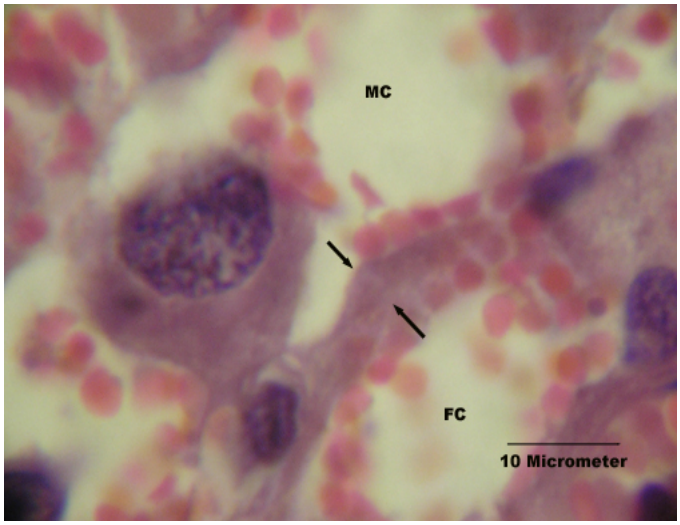


**Figure II: Section of mouse placenta (case S 45b), showing thickened maternal fetal barrier (arrow) in the labyrinthine layer. Fetal capillary (FC) and maternal sinusoid (MC). Haematoxylin and eosin stain. Photomicrograph. Bar, 10 µm**

d. **Distance between mononuclear trophoblast cells**: Large mononuclear cells were seen projecting into the lumen of maternal sinusoids. The mean distance between the nuclei of these cells showed a statistically significant increase in the Smoke group (38.36±2.05µm) as compared to their counterparts in the Control group (33.48±1.00µm) with a P value of 0.01. However, this increase in distance was observed only on moderate exposure to smoke. Moreover, this distance was not statistically affected (P=0.17) in the Smoke plus Vitamin group (33.58±1.37µm) as compared to the Control 19 dpc placentae(Table I).

**Table I: Thickness of Maternal Fetal Barrier and the distance between Mononuclear Trophoblast cells in 19 DPC mouse placentae**

| | | Maternal Fetal Barrier µm Mean ± SE | Distance between Mononuclear cells µm Mean ± SE |
|---|---|---|---|
| Control group | n= 14 | 1.31±0.04 | 33.48±1.00 |
| Smoke Group (pooled mild and moderate smoke) | n=12 | 2.21±1.35 | 38.36±2.05 |
| Smoke Plus Vitamin Group (pooled mild and moderate smoke) | n=14 | 1.66±0.09 | 33.58±1.37 |
| Smoke Group (Mild Smoke) | n=5 | 1.75±0.06 | 36.05±1.24 |
| Smoke Group (Moderate Smoke) | n=7 | 2.54±0.11 | 41.01±3.01 |
| Smoke Plus Vitamin Group (Mild Smoke) | n=5 | 1.43±0.9 | 31.91±0.35 |
| Smoke Plus Vitamin Group (Moderate Smoke) | n=9 | 1.70±0.10 | 31.90±0.35 |

*SE: Standard error*
*n : Number*

# Discussion

Evidence is mounting to suggest that direct relationship exist between gestational environment (nutrition, chemical exposure, stress, and exercise) and elevated risk of disease in the newborn[16]. Prenatal exposure to cigarette smoke exposes the placenta and indirectly the fetus to free radicals which are responsible for producing oxidative injury. This view is supported by studies which have shown an increase in the expression of antioxidant mechanism (heme oxygenase) in the smoke exposed placenta.[17]

The present study of the mouse placenta confirmed that the innermost layer towards the fetal side of the placental disk was the labyrinthine layer, the architecture of which was in accordance with previous electronmicrographic observations that the mouse placenta has a hemotrichorial maternal fetal barrier. This barrier is made up of two layers of syncytiotrophoblast cells and large mononuclear trophoblast cells protruding into the maternal sinusoids[6]. In mice the fetal capillaries and the maternal lacunae of the labyrinthine layer form a large contact surface for exchange of products between the mother and fetus, and so this layer is functionally homologous to the area of human placenta which contains chorionic villi and the intervillous space.[18]

However, in the present study, the smoke exposed placentae showed a significantly thicker barrier even on mild exposure to smoke as compared to their counter parts in the control group. This thickening was related to the extent of smoke exposure and a highly significant increase in thickness was seen when the exposure increased from mild to moderate degree. This is also in accordance with a human study in which the effects of passive smoking on human placenta showed that the trophoblastic basement membrane and thus the maternal fetal barrier were thicker in the group exposed to smoke[9]. Similar, other human studies have also shown that the mean thickness of the trophoblast component of the villous membrane increased in the smoke exposed plcentae. [19]

The thickness of the villous membrane is known to be an important factor in determining the morphometric diffusing capacity of the placenta at term[20]. Therefore, when there is impairment of placental barrier, the transport between mother and fetus is hampered [21]. The fetuses exposed to smoke suffer hypoxic stress, and the morphological changes observed compromise rather than assist, transplacental oxygen transfer. This is in marked contrast to the adaptive changes seen in pregnancies associated with hypoxia related to high altitude[22,23] and pregnancies associated with protein malnutrition which are characterized by thinning of the villous membranes.[24] This further suggests that factors other than hypoxia might be compromising the fetoplacental unit. Starvation, which is also associated with reduced fetal weights, is characterized by thickening of the barrier. In these cases, the reduction in weight indicates functional impairment beyond what would be expected by food restriction alone [25] and, the thickening of the barrier may also be partly responsible for the decreased fetal weight.

In the present study, the distance between nuclei of mononuclear trophoblast cells in the maternal sinusoids was seen to increase upon exposure to smoke. This increase in distance may be a compensatory mechanism of slightly decreasing the thickness of the maternal fetal barrier secondary to the overall thickening in the Smoke exposed group.

The present study shows that supplementation with vitamins significantly ameliorated the harmful effects of mild and moderate exposure to cigarette smoke on the mouse placenta; however complete reversion was not achieved. This might be possible with

increased doses of antioxidants which could be dealt with in future studies.

## Conclusion

Prenatal exposure to cigarette smoke which contains free radicals and other toxic chemicals has adverse effects on the histology of mouse placenta including a thickening of the maternal fetal barrier. The administration of antioxidants ameliorated these harmful effects significantly. Therefore it can be assumed that some of the toxic effects of tobacco smoke are due to oxidative injury produced by free radicals which can be prevented to some extent by appropriate doses of antioxidants.

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