The Effects of Passive Smoking on the Placental Barrier: A Light Microscopic Study
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Abstract:
Passive exposure to tobacco smoke causes ultrastructural changes in placental vasculature. It was endeavoured to observe under the light microscope, the endothelial cells of the capillaries and to look for microscopic changes in the vasculosyncytial membranes in the terminal villi of placentae of passive smokers exposed largely to bidi smoke. To study effect of passive smoking on placental vasculature a total of 150 placentae were selected from healthy full term gravidas with no history of tobacco use. They were divided into two group viz; passive smokers (n=87) and non passive smokers-control (n=63). Three micron thick sections were cut, stained and examined under light microscope. The study revealed that endothelial cells of stromal capillaries of the placental villi were oedematous resulting in irregular capillary lumina (p=0.0007) and vasculosyncytial membrane was significantly absent in terminal villi of passive smokers in comparison to control (p=0.0000).

Key Words: Passive smoking, Placentae.

Introduction:
Bidi smoking is the most popular form of smoking tobacco in India followed by cigarette smoking (Gupta & Asma, 2008). Bidis or beedis or biris are slim, hand rolled, unfiltered form of smoking tobacco.

According to the National Family Household Survey-3, conducted in India in the years 2005-2006, 32.7% of males and 1.4% of females smoke bidis or cigarettes in the age group of 15-54 years (WHO, 2008). This means that approximately 30 % of women in India in the reproductive age group are passive smokers, exposed largely to bidi smoke.

Passive smoking is the involuntary consumption of smoked tobacco. There are two forms of passive smoking. “Second–hand smoke” is passive consumption, when the burning end is still present. Environmental tobacco smoke or “third-hand smoke”, is the consumption of smoke that remains after the burning end is extinguished (Tobacco smoking, 2010).

Due to its negative implications, this form of consumption has played a central role in the regulation of tobacco products. Asmussen (1977; 1978) conducted electron microscopic studies on human placentae of fullterm gravida. In the smokers he found a broadening of the basement membrane of the placental villi, increased collagen content of the villi, decreased vascularity, and intimal changes in the villous capillaries and arterioles with pronounced intimal oedema. Rath et al (2011) studied the morphology and did morphometric analysis of stromal capillaries in full term placental villi of smoking mothers and revealed that endothelial cells of placental villi of stromal capillaries were oedematous, and the cytoplasm was rich in dilated endoplasmic reticulum, mitochondria, fibrils and fine filaments in active as well as passive smokers. Morphometric analysis showed a decrease in the perimeter and area of the stromal capillaries, but an increase in number of capillaries per villus in passive smokers. Passive exposure to tobacco smoke, besides having adverse effects on the respiratory and cardiovascular system of the expectant mother, can affect placental tissue directly or indirectly by causing vasoconstriction of maternal vessels.

There is a relative paucity of research on effects of passive smoking on the placenta. Therefore, the present study was undertaken to observe the effect of passive smoking on stromal capillaries of terminal villi of placentae by the light microscope.

Material and Methods:
One hundred and fifty placentae were collected immediately after delivery (36-42 weeks of gestation) from the Department of Obstetrics and Gynaecology, Subharti Medical College, Meerut. Out of these 150...
placentae, 63 were collected from healthy gravidae with no history of exposure to tobacco smoke, or consumption of tobacco in any form, and were labelled as controls. Eighty seven placentae were collected from passive smokers mostly exposed to bidi smoke. A gravida exposed to 1 smoker smoking over a packet a day, or 2 or more smokers combined smoking at least 10 cigarettes or beedis a day, was labelled as passive smoker (Luciano et al, 1998).

Immediately after the expulsion of the placenta, the umbilical cord was cut 5 cms away from the site of the insertion, and membranes were trimmed. The clots were removed from the maternal surface and it was gently blotted dry with filter paper. This was followed by immersion & fixation of the placentae in 10% buffered formalin (pH 7).

Exclusion Criteria:
1. Gravida with IDDM or NIDDM, gestational diabetes, essential hypertension or pregnancy induced hypertension.
2. Gravida who actively consumed tobacco in any form; (e.g. cigarettes, bidis, paan, zarda, as tooth powder, hookah, etc)
3. Gravida with exposure to hookah smoke as it is difficult to quantify tobacco used in such cases.

From the maternal surface of the placental disc, 1 cm thick sections, one from the centre and one from the periphery, were taken and fixed in neutral buffered formalin for 24 hours. Routine processing was carried out for preparation of paraffin blocks. Three micron thick sections were cut and stained with Haematoxylin & Eosin. Histological parameters were observed under trinocular light microscope with attached digital camera (Nikon Eclipse E-200), and photomicrographs were taken with the help of Q capture software.

Capillary lumen and vasculosyncytial membrane were studied in detail; a total of 100 villi per slide were observed.

Results:
The capillary lumina of the terminal villi were irregular in approximately 9% of passive smoking group (Table I). An irregular capillary lumen was characterised by protrusion of endothelial cells into the lumen (Fig. I).

Approximately 36% of terminal villi in the passive smoking group had absence of vasculosyncytial membrane (Fig. III). The vasculosyncytial membrane appeared as a thin amphophilic line formed by cytoplasm of syncytiotrophoblast, trophoblastic basement membrane, capillary basement membrane and a thin layer of intervening stroma (Fig. II).

Table I: Table showing mean and standard deviation in the 2 groups, and probable values of unpaired t-test for the different histologic parameters.

<table>
<thead>
<tr>
<th>Histopathological parameter</th>
<th>Passive smokers group mean± SD</th>
<th>Control group mean± SD</th>
<th>Probable values of t-test unpaired</th>
</tr>
</thead>
<tbody>
<tr>
<td>Irregular capillary lumen</td>
<td>8.74±8.04</td>
<td>5.28±4.01</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Absence of vasculosyncytial membrane</td>
<td>35.62±32.82</td>
<td>15.68±15.86</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Discussion:
As placenta is the most important organ between mother and fetus during prenatal period, the toxic effect of nicotine may be manifested in it (Lieberman et al, 1994). It is likely that chronic exposure to tobacco constituents in early pregnancy can affect placental development directly or indirectly by reducing blood flow, which creates a pathologically hypoxic environment.

Fig. I: Photomicrograph of placenta of passive smoker group showing irregular capillary lumen (C) with protrusion of endothelial cell (E; H&E, 400X).

An irregular capillary lumen was found in 8.74±8.04 per 100 terminal villi of passive smokers in the present study as compared to 5.28± 4.01 per 100 terminal villi in the control group. Amsussen (1977;1978) observed intimal changes in villous capillaries and arterioles with pronounced intimal oedema in smoker’s placentae. Burton et al (1989)
error in their study due to under or over distension of the fetal vessels should be considered.

The vasculosyncytial membrane was absent in 35.62±32.82 per 100 terminal villi of passive smokers as compared to 15.68±15.86 per 100 terminal villi of the control group of the present study. The vasculosyncytial membrane (VSM) is considered to be the principal site for gaseous transfer. It is the area of cytoplasm of syncytiotrophoblast in close approximation with capillary endothelium, there being minimal stroma between capillary and trophoblast basement membranes. Similarly, Van der Veen & Fox (1982) also found a paucity of VSM in terminal villi of women who smoked.

The placenta serves as an important conduit of nutrients and some toxic metabolites like nicotine and carbon monoxide from the mother to the developing fetus. Nicotine is a powerful vasoconstrictor (Liebhart, 1974). Passive exposure to tobacco smoke causes uterine vasoconstriction, which in turn results in under perfusion of the placenta. Under perfusion of the placenta results in placental hypoxia, which in turn may be responsible for majority of the changes observed. Endothelial cells exposed to anoxic stress, show increase in levels of nitric oxide (NO) and the expression of inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2) and tumour necrosis factor-\(\alpha\) (TNF \(\alpha\)), molecules associated with endothelial dysfunction and vascular diseases (Palmieri et al, 2012).

Vascular changes in terminal villi could well be an indication of similar changes having occurred in other vessels of the newborn, which could be a harbinger of sequelae like atherosclerosis later in life (Asmussen, 1979).

In the present study oedema of endothelial cells was observed resulting in irregular capillary lumina, and absence of vasculosyncytial membrane in a significant number of villi in passive smokers even at the light microscopic level.

Therefore, in view of these observations it would be prudent to advise family members and other close associates of an expectant mother to abstain from smoking in the interests of the unborn child.

References:


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