

Placental Vasculosyncytial Membrane in Tobacco exposed Indian Mothers: A quantitative and ultra structural study.

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Abstract: A comparative electron microscopic study was conducted to observe the effect of tobacco on the ultrastructure of placental vasculosyncytial membrane (VSM) during pregnancy. A study group comprising of 6 active smokers, 11 passive smokers and 8 tobacco chewers were morphometrically analysed against a control group of 25 placentae collected from non tobacco consuming mothers. A significant thickening at sites of VSM with a reduction in their number per tertiary villi, was observed in the term placentae of all 3 sub groups of tobacco consumers in contrast to the control placentae. Significant trophoblastic basement membrane (TBM) thickening and cytotrophoblastic hyperplasia was also noticed in placentae of tobacco consuming mothers. All these findings suggest a possible impairment of materno fetal transfer of nutrients and gases across the sites of VSM; thus resulting in deleterious fetal outcomes.

INTRODUCTION

Placenta undoubtedly plays an essential role in fetomaternal exchange during pregnancy at the level of the tertiary villi across a selective placental barrier which comprises of (a) trophoblast i.e. syncytiotrophoblast and occasional cytotrophoblast with trophoblastic basement membrane (b) connective tissue stroma and (c) endothelial cell and its basement membrane. In tertiary villi of term placentae, the fusion of fetal capillaries and the thin overlying syncytiotrophoblast forms an area called vasculosyncytial membrane (VSM) which is believed to be vital in gaseous exchange between maternal and fetal blood. Considerable biological evidence supported by Viogt et al and Genbecav et al suggests that smoking contributes to adverse pregnancy outcomes like premature deliveries, fetal growth restriction and low birth babies all of which are effects of resultant fetal hypoxia due to nicotine, cadmium and polyaromatic hydrocarbons on components of VSM thus comprising fetomaternal exchange. Ultrastructural changes of VSM of smokers studied by Vander Velde et al and Demir et al show a decrease in number of VSM and basement membrane thickening as pregnancy proceeds. Majority of such changes are reported in active cigarette smokers in the western population with very few studies reported in Indian women where tobacco is mostly consumed as bidi either passively and by ingestion. Thus the present study is an attempt to observe ultrastructural changes of VSM in Indian smokers, and to comparatively analyse the severity of changes in passive smokers and tobacco chewers in respect to active smokers.

MATERIALS AND METHODS

A total number of 50 placentae were collected immediately after delivery from the labour room of Lady Hardinge Medical College and Smt Sucheta Kriplani Hospital, New Delhi. All placentae belonged to primigravid mothers of age 20-25 years and between 32-40 weeks of gestation. The placentae were divided into 2 groups:

1. Control group (25 Placentae)- belonged to non tobacco consuming healthy mothers who neither smoke nor were exposed to passive smoking from husband or close relative.
2. Study group (25 placentae)- belonged to mothers consuming tobacco in any form and were further grouped as:-
 - (a) Active smokers (6 placentae)- who consume 30 or more

cigarettes or bidis per day.

(b) Passive smokers (11 placentae)- whose husbands or close relative consumed 30 or more cigarettes or bidis per day.

(c) Tobacco chewers (8 placentae)- who consumed tobacco products by chewing.

Soon after collection the placentae were fixed by perfusion with 2% of 0.1M glutaraldehyde (pH-7.4) and tissue about 1-2 cm was collected and post fixed in 1-2 % osmium tetroxide. Routine processing was further carried out embedding was done in Spurr's resin. Semithin sections were cut on a LKB ultramicrotome and stained with toluidine blue. Selected areas were subjected to electron microscopy and stained with lead citrate and uranyl acetate, and examined under Hitachi TEM-H 7500. Ultrastructural of VSM was observed in both control and study group and morphometric study was conducted with the help of SIS 3.0 software (software imaging system, GmbH, Germany). All data were statistically analysed.

RESULTS

A) Control placentae (non smokers)

In placentae of non smokers, vasculosyncytial membranes (VSM) were seen in various sections of tertiary villi, where the fetal capillary was found bulging into the syncytiotrophoblast so that there seemed to be an apparent fusion of trophoblastic and endothelial cell basement membrane with very little or absence of connective tissue stroma in between them. At this site the syncytiotrophoblast in comparison to other areas of terminal villi was greatly thinned out to form syncytial lamellae and numerous microvilli of varying height were found at its free surface. The number of VSM per villous profile was recorded to be 3.14 ± 2.29 . The thickness of VSM and trophoblastic basement membrane (TBM) in nonsmokers placentae was found to be $6.44 \pm 3.14 \mu\text{m}$ and $0.27 \pm 0.14 \mu\text{m}$ respectively. The cytoplasm of cytotrophoblast was found to be only occasionally present and the number was 0.90 ± 1.02 . The cytoplasm of both syncytiotrophoblast and endothelial cells showed organelles like SER, lysosomes, mitochondria, fibrils and filaments at sites of VSM. (Fig 1 and 3)

B) Study group placentae (smokers)

In active smokers, the numbers of VSM per villous were decreased

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to 2.82 ± 1.74 in comparison to control. The areas of VSM appeared to be thicker due to increased thickness of trophoblastic and endothelial cell basement membrane. The thickness of VSM and TBM were $7.97 \pm 4.38 \mu\text{m}$ and $0.34 \pm 0.21 \mu\text{m}$ respectively. The microvilli on the surface of syncytiotrophoblast were found to be fewer, blunt tipped, rounded or occasionally broken at places. While cytotrophoblast number increased to 3.50 ± 1.96 due to prominent hyperplasia. Considerable amount of connective tissue stroma intervened at sites of VSM. The lumen of the fetal capillaries appeared constricted due to endothelial cell protrusion into it. Unlike control group cytoplasmic organelles like ribosomes, dilated rER, mitochondria, microfilaments and fibrils were abundantly found in the cytoplasm of syncytiotrophoblast and endothelial cells. (fig 2,4 and 5). In the placentae of passive smokers the number of VSM was 2.84 ± 0.24 , the thickness of VSM and TBM were found to be $7.86 \pm 5.21 \mu\text{m}$ and $0.88 \pm 0.36 \mu\text{m}$ respectively. Here the microvilli were found to be fewer and reduced in height. The cytotrophoblast number increased to 2.89 ± 1.11 . Rest of the EM changes at sites of VSM in passive smokers were similar to that of active smokers. In tobacco chewers, the number of VSM, thickness of TBM and VSM were recorded as 2.97 ± 0.25 , $0.37 \pm 0.22 \mu\text{m}$ and $6.61 \pm 3.82 \mu\text{m}$ respectively. Number of cytotrophoblast noted were 2.45 ± 0.76 . The ultrastructural changes of other components of VSM were not in comparison to non smokers. On analyzing the above data statistically, the changes in the number of VSM per villous profile was found to be non-significant in all 3 study group as compared to the control group. The thickness of VSM was significantly altered in active and passive smokers when compared to non smokers; but the changes in tobacco chewers was insignificant in all three study groups (active smokers, passive smokers and tobacco chewers) when compared against the control placentae. The morphometric and statistical analysis of the above data is tabulated in table 1.



Figure 1- VSM (white line) in control placenta showing syncytiotrophoblast (S), trophoblast basement membrane (TBM), stroma (St), endothelial basement membrane (EBM), endothelial cell (EC) capillary (C) and intervillous space (IVS). 4800 X.

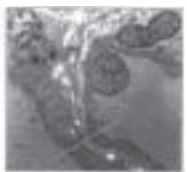


Figure 2- VSM (white line) in active smokers placenta showing reduced number and blunt microvilli (Mv) at the free surface of Syncytiotrophoblast (S). The trophoblastic basement membrane (TBM), stroma (st), endothelial cell basement membrane (EBM), intervillous space (IVS) and endothelial cell (EC) protrusion into fetal capillary (C) are also seen. 15000X.

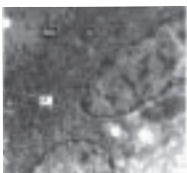


Figure 3- Syncytiotrophoblast in control placenta showing microvilli (MV) at the free surface, nucleus (N) along with smooth endoplasmic reticulum (sER) and lysosomes (L) in its cytoplasm. 10000X



Figure 4- Nucleus (N) and cytoplasm of cytotrophoblast (Cyt) showing mitochondria (M) and rough endoplasmic reticulum (rER). Thickening of trophoblastic basement membrane (TBM) is also seen. 20000X

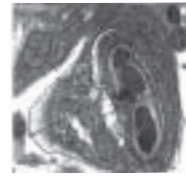


Figure 5- Electromicrograph of placental villi showing the procedure for the measurement of different parameters for morphometric analysis in placenta of study group.

Table 1: Comparative morphometric and statistical analysis in control and study group.

| Parameter | Non-smokers (n=25) | Active smokers (n=6) | Significance | Passive smokers (n=11) | Significance | Tobacco chewers (n=8) | Significance |
|--------------------------------------|--------------------|----------------------|--------------|------------------------|--------------|-----------------------|--------------|
| No. of VSM per villous profile | 3.14 ± 2.29 | 2.82 ± 1.74 | Ns (p>0.01) | 2.84 ± 0.24 | Ns (p<0.01) | 2.97 ± 0.25 | Ns (p>0.01) |
| Thickness of VSM (in μm) | 6.44 ± 3.14 | 7.97 ± 4.38 | P<0.05 | 7.86 ± 5.21 | P<0.05 | 6.61 ± 3.82 | ns |
| Thickness of TBM (in μm) | 0.27 ± 0.14 | 0.34 ± 0.21 | P<0.005 | 0.88 ± 0.36 | P<0.00001 | 0.37 ± 0.22 | P<0.00005 |
| No. Of cytotrophoblast | 0.90 ± 1.02 | 3.50 ± 1.96 | P<0.00005 | 2.80 ± 1.11 | P<0.00001 | 2.45 ± 0.76 | P<0.00001 |

DISCUSSION

The vasculo syncytial membranes were defined by Fox and Blanco as extremely thin syncytiotrophoblastic lamellae which are directly apposed sinusoidally enlarged fetal capillaries. These areas are supposed to be specially involved in difusional transfer of gases and water and facilitated transfer of glucose during pregnancy as reported by Benirshke and Kaufmann⁶.

Vander Veen et al and Vandervelde et al reported a paucity of VSM as well as an apparent increase in their thickness due to irregular thickening of trophoblastic and endothelial cell basement membrane at these sites in smokers placentae. But these studies were only qualitative. Quantitative data were provided by Vandervelde and Treffers⁸, indicating an increase in thickness of $0.14 \mu\text{m}$ at VSM, in women who smoked more than 25 cigarettes a day. In another morphometric study by Burton et al⁹ the mean thickness of VSM measured in nonsmokers and smokers were reported to be $3.61 \mu\text{m}$ and $4.16 \mu\text{m}$ respectively. In our present study the sites of VSM in smokers placentae were also found to be insignificantly ($p < 0.05$) thickened in both active ($7.97 \mu\text{m}$) and passive smokers ($7.86 \mu\text{m}$) as compared to non smokers ($6.44 \mu\text{m}$). Such changes were attributed to placental ischemia or hypoxia consequent upon nicotine induced constriction of uterine vessels and accumulation of toxic constituents of tobacco. Such ultrastructural changes were also reported by Kurfukar et al¹⁰ and Pietryga et al¹¹ in placentas of prolonged pregnancies, PIH and gestational diabetes all of which result in fetal hypoxia. Dimitrovska¹² reported a low number of VSM to be suggestive of either villous immaturity or villous regressive changes and found mature babies with placentas of low VSM suffered from high incidence of hypoxic conditions. Such changes can also be induced in experimental animals by exposure to carbon monoxide or by exposing human placental tissue to hypoxic perfusion as suggested by Bachmaier et al¹³.

Microvilli at the free surface of syncytiotrophoblast represent an area of strong feto maternal contact and studies by Vanderveen and Fox⁸ and Demir et al⁴ revealed an abnormality in their structure in smokers placentae. Similarly in our study, less number and reduced heights of microvilli were observed in all three groups of tobacco users, the changes being more prominent in passive smokers. This difference may be due to the fact that passive smokers may be exposed to more tobacco smoke than they have actually disclosed. Since smoking is a social stigma in India it might have prompted the passive smoking mothers to mention lesser number of bidi or cigarettes. Since the main function of microvilli is to increase the total villous surface area for absorption and transmembranous transfer process, their reduced number in tobacco users may be responsible for insufficient maternofetal transfer of nutrients and gases which probably

explain the incidence of low birth weight babies born to this group. Cytotrophoblastic hyperplasia had been observed to be significantly increased in placental villi of smoking mothers by Naeye¹⁴. This was explained to be the consequence of degenerative changes in the syncytiotrophoblast which induces cytotrophoblast differentiation and ultimately syncytial fusion. In the present study such hyperplasia was also observed which might be essential for maintenance of functional and structural integrity of the overlying syncytial layer. Mayhew et al¹⁵ observed similar cytotrophoblastic hyperplasia of mothers from several pathological causes related to hypoxia such as anaemia and pregnancies at high altitude. So it can be concluded that reduced supply of oxygen damages the syncytiotrophoblastic layer which in turn induces cytotrophoblastic proliferation.

Thickened trophoblastic and endothelial cell basement membrane at sites of VSM as found in our study were reported by Demir et al. since both TMBM and EBM act as important components of the filtration barrier at VSM. Their apparent thickening in smokers placenta reduces blood flow to fetal side thus hampering fetomaternal transport.

Cytoplasmic organelles like numerous mitochondria and dilated rER were observed in the cytotrophoblast in active and passive smokers and also tobacco chewers. Such observations were supported by studies of Kaufmann¹⁶. Presence of mitochondria and rER are speculated to be involved in more complex and active maternofetal transfer mechanisms including metabolism and synthesis of proteins and lipids and it may be interpreted as a sign of increased energy requirements, since mitochondria is known to be the site for oxidative phosphorylation and ATP synthesis. Naeye¹⁴ have studied the stromal aspect in case of smokers placenta, and reported stromal fibrosis and increased collagen content. In our ultrastructural study, we noticed an increased intervention of stromal tissue at sites of VSM, but no remarkable difference was noticed in all three subgroups of smokers placenta.

The observed changes in almost all components of VSM in active, passive smokers and tobacco chewers in the present study are suggestive of the fact that transfer of essential nutrients, fluids and gases were compromised between the mother and fetus resulting in adverse fetal outcomes like IUGR, low birth weight babies and still births. But it is also known that all babies born to tobacco using mothers do not suffer from such health hazards. This may be supported by the fact reported by Kameya et al¹⁷ and Scheibler et al¹⁸ that thickened VSM are known to possess hydrolytic enzymes like alkaline phosphatase, 5 nucleotidase and ATPase which are involved in active transfer of nutrients. Thus the increased thickness of VSM in placental villi of tobacco users may compensate the fetal intake of important gases, water, amino acids, electrolytes etc. by actively transporting them from the maternal side, whose transfer to the fetus by the process of passive diffusion is otherwise reduced due to decrease in number of vasculo syncytial membrane (VSM). Further molecular analysis of the components of VSM, may be beneficial to support this study.

CONCLUSION

The ultrastructural changes observed in this study on the components of placental vasculosyncytial membrane support the fact that diffusional transfer of gases and nutrients from mother to fetus is highly impaired in tobacco consuming mothers. Thus tobacco consumed in any form either by active and passive smoking or by chewing tobacco products is greatly injurious to both fetus and newborn resulting inevitable adverse sequelae like IUGR, LBW babies etc, which can be safely avoided by abstaining from consuming tobacco during pregnancy.

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