Original Article

The Effects of Passive Smoking on the Human Placenta: A Gross and Microscopic Study

Srividya Sreenivasan[1,2], Satyam Khare[1]

[1]Department of Anatomy, Subharti Medical College, Meerut, Uttar Pradesh, [2]Department of Anatomy, Dr. DY Patil Medical College, Navi Mumbai, Maharashtra, India

Abstract

Introduction: Passive smoking is an established health hazard. Placenta is the main conduit of both nutrients and toxins to the fetus. This study aims to study the effect of passive smoking on the gross and microscopic changes in the placenta among passive smokers. **Material and Methods:** Gross and microscopic examination was done of the 150 term placentae donated to the department of anatomy; 63 were from gravidas with no history of exposure to tobacco smoke and labeled as controls, whereas 87 placentae were collected from passive smokers. **Results:** We observed a significant increase in the calcification of fetal surface of the placentae in our cohort (approximately 16% as compared to approximately 13% in the control group). Syncytial knots were found in 43% of the terminal villi in our cohort. Fibrinoid degeneration was observed in approximately 7% of the terminal villi of passive smokers. Hyperplasia of cytotrophoblast was observed in approximately 30% of the terminal villi in our cohort. 14% of the terminal villi of passive smokers had thickening of subtrophoblastic basement membrane. 9.47% of the terminal villi in the passive smoking group were avascular. These findings were statistically significant. **Discussion and Conclusion:** Passive smoking causes changes in the placenta at the gross and microscopic level. The gross and histologic changes seen in the placenta in this study are known to be associated with adverse fetal outcome. The changes in the placenta can serve as a means of understanding the mechanism of the fetal morbidity due to active or passive smoking.

Keywords: *Histology, passive smoking, placenta*

Introduction

Smoking is an established health hazard. Passive smoking is the involuntary consumption of smoked tobacco. It is easy to miss and difficult to quantify. Luciano *et al.* defined passive smokers as having significant exposure to cigarette smoke either at work or at home, i.e., exposure to one smoker smoking over a pack a day, or two or more smokers smoking 10 cigarettes per day.[1] In India, about 25% of males and 4% of females smoked tobacco as of 2011.[2] Hence, expectant mothers are more likely to be passively exposed to cigarette smoke than be an active smoker. A gravida exposed to passive cigarette smoke has a greater probability of having preterm delivery, intrauterine growth retardation, low birth weight, and still-birth.[3] The placenta is a conduit for nutrients and toxins to the fetus. The purpose of this study was to ascertain if passive smoking caused any changes in the placenta at the gross and microscopic level.

Material and Methods

A total of 150 placentae were collected immediately after delivery (36–42 weeks of gestation). 63 placentae were collected from parturients with no history of exposure to tobacco smoke, or consumption of tobacco in any form, and labeled as controls, whereas 87 placentae were collected from passive smokers. A gravida exposed to one smoker smoking over a pack of cigarettes (pack of 10) or a bundle of bidis (bundle of 25) a day, or two or more smokers combined smoking at least 10 cigarettes or 25 bidis a day, was labeled as a passive smoker.[1]

Exclusion criteria

1. Gravida with insulin-dependent diabetes mellitus (IDDM) or non-IDDM, 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.)

How to cite this article: Sreenivasan S, Khare S. The effects of passive smoking on the human placenta: A gross and microscopic study. J Anat Soc India 2019;68:34-8.

Address for correspondence:
Dr. Srividya Sreenivasan, Department of Anatomy, Dr. DY Patil Medical College, Nerul, Navi Mumbai - 400 706, Maharashtra, India.
E-mail: srividyasreenivasan@yahoo.com

Access this article online

Website: www.jasi.org.in

DOI: 10.4103/JASI.JASI_5_19

Quick Response Code:

3. Gravida with exposure to hookah smoke as it is difficult to quantify tobacco used in such cases
4. Gravida working in bidi-making industry.

Immediately after the expulsion of the placenta, the umbilical cord was cut 5 cm away from the site of insertion, and the membranes were trimmed. The clots were removed from the maternal surface, and it was gently blotted dry with a filter paper. A gross examination was done under the following heads:

1. The fetal surface was observed for the presence of discoloration, engorgement of blood vessels, calcification, and hematoma
2. The maternal surface was screened for the presence of hemorrhagic and ischemic infarcts, compression, and calcification.

This was followed by immersion fixation of the placentae in 10% buffered formalin (pH-7).

From the maternal surface of the placental disc, two sections, i.e., one from the center and one from the periphery of about 1-cm thickness, were taken and fixed in neutral-buffered formalin for 24 h. Routine processing was carried out for the preparation of paraffin blocks. Three-micron thick sections were cut. These were stained with hematoxylin and eosin (H and E) stain and periodic–acid Schiff (PAS) stain. Histologic parameters were observed under a trinocular light microscope with an attached digital camera (Nikon Eclipse E200), made in Japan and photomicrographs were taken with the help of EZCAPTURE software, version 2.5, (AVermedia Technologies Inc, New Taipei City, Taiwan). One hundred terminal villi were examined for each of the following histological parameters in each slide.

1. Number of villi with syncytial knots
2. Number of villi with deposition of fibrin
3. Number of villi with hyperplasia of cytotrophoblast
4. Number of villi with thick subtrophoblastic basement membrane (TBM)
5. Number of avascular villi.

While most of the changes were well appreciated with H and E staining, the thickness of the basement membrane was studied better on sections stained with PAS.

The mean and standard deviation were calculated for each of the histological parameters for changes observed per 100 villi in the cohort and control group. Unpaired *t*-test was performed to determine significance, with the $P \leq 0.05$. Microsoft Excel was used for statistical and graph work.

Results

We observed a significant increase in calcification of the fetal surface of the placentae in our cohort [Figure 1] (16.09% as compared to 12.69% in controls), with *Z* value being infinity and level of significance >1.96. No other significant gross abnormality was noted in the placentae of our passive smokers, nor in the control group.

The percentage of terminal villi of passive smokers having syncytial knots [Figure 2] was 43.47%, while only 27.07% of the terminal villi in the control group had the same finding, $P = 0.0000$ [Table 1].

Fibrinoid degeneration was observed in 7.24% of the terminal villi of passive smokers [Figure 2] ($P = 0.000127$). Villi had a homogenous pink appearance in the areas of fibrinoid deposition. The cytotrophoblasts were occasionally present under the syncytiotrophoblast. They were present above the TBM. They had an intensely basophilic cytoplasm, with a round or oval vesicular nucleus occupying most of the cell. Since cytotrophoblasts are usually not seen in term placentae under a light microscope, the presence of a cytotrophoblast in a terminal villus was considered as hyperplasia of the cytotrophoblast. Hyperplasia of the cytotrophoblast was observed in 29.97% of the terminal villi in passive smokers, as compared to 12.38% of villi in controls [Figure 3] ($P = 0.0000$). 13.64% of the terminal villi of passive smokers had thickening of sub-TBM as compared to 4.88% of the terminal villi in our control group ($P = 0.0000$). This was best observed in

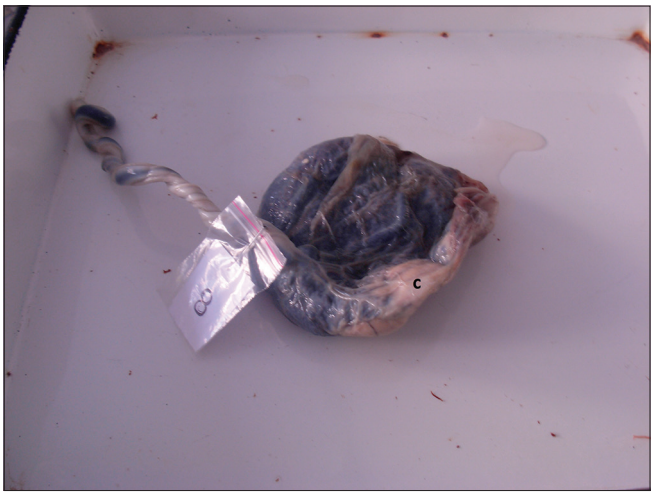

Figure 1: Fetal surface of the placenta of passive smoker showing calcification (c)

Table 1: Mean and standard deviation of different histopathological parameters in the two groups

Histopathological parameters (per 100 villi)	Mean±SD	
	Cohort	Control
Syncytial knots (number of knots)	43.47±22.52	27.07±17.2
Fibrinoid degeneration (number of villi)	7.24±8.01	3.36±3.75
Hyperplasia of cytotrophoblast (number of villi)	29.97±22.87	12.38±10.9
Thick subtrophoblastic basement membrane (number of villi)	13.64±13.17	4.88±3.75
Avascular villi	9.47±20.44	3.8±9.84

SD=Standard deviation

our PAS-stained sections [Figure 4]. The thickness of the basement membrane was not uniform in a given villus.

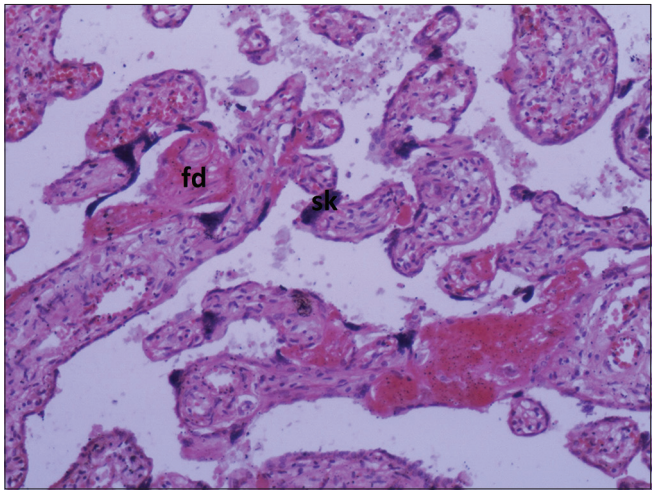

Figure 2: Photomicrograph of the placenta of passive smoker showing fibrinoid degeneration (fd) and syncytial knots (sk) (H and E, ×100)

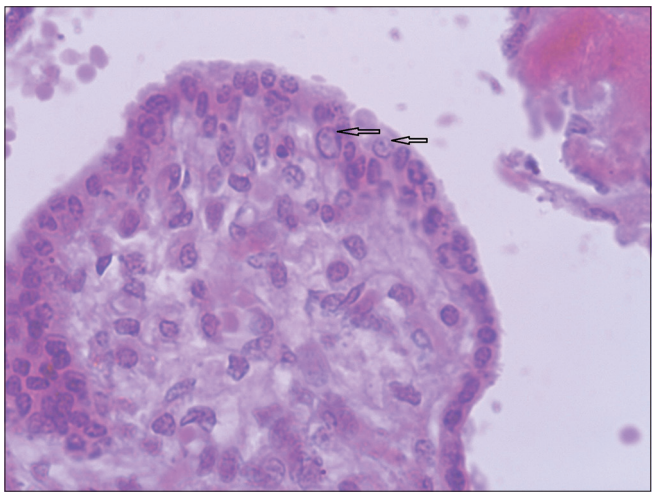

Figure 3: Photomicrograph of the placenta of passive smoker showing avascular villus, arrows indicate cytotrophoblasts (H and E, ×400)

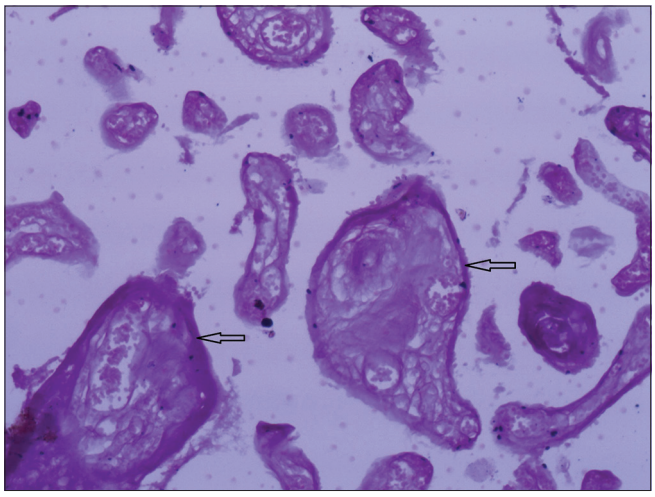

Figure 4: Photomicrograph of the placenta of passive smoker showing thickened basement membrane indicated by arrows (PAS, ×100)

9.47% of the terminal villi in the passive smoking group were avascular as compared to 3.8% of terminal villi in the control group (P = 0.02624). Those villi where a capillary lumen could not be demonstrated were labeled avascular [Figure 3].

Discussion

The majority of our study group comprised of homemakers living in joint families. Ninety percent of our cohort was exposed to bidi smoke; the remaining 10% was exposed to cigarette smoke. Their combined exposure from 1 to 2 contacts was by and large 1–2 bundles per day.

We observed a significant increase in calcification of the fetal surface of the placentae in our cohort (approximately 16% as compared to approximately 13% in the control group) [Figure 1]. Christianson had reported an increased prevalence of calcification in placentae of smokers.[4] The striking similarity of placental changes in women living at high altitudes and smokers has given strong support to the hypothesis that hypoxia could contribute to the differences observed. Our findings on gross examination of the placenta are at variance with Rath *et al*., who found no gross abnormality, namely calcification or infarction of placental tissue, neither in controls nor in the passive smokers.[5] The differences in our observations could be explained by the fact that our study group was from a rural area as compared to their urban population. The calcification seen could possibly be due to the influence of some environmental factors prevailing in rural Western Uttar Pradesh.

Some (pre-) apoptotic nuclear aggregates of syncytiotrophoblast are sequestered in the syncytial knots, extruded as trophoblast fragments into the intervillous space, and then deported into the maternal circulation to be phagocytosed at extraplacental sites. We have observed a significant increase in syncytial knots (43.47% ± 22.52%) in the placentae of passive smokers as compared to controls [Table 2]. Rath *et al*. in 2001 had reported similar

Table 2: Probable values of unpaired *t*-test between cohort and controls for different histological parameters

Histopathological parameters (per 100 villi)	Probable values of *t*-test unpaired	Significance of *P*
Syncytial knots (number of knots)	0.0000	<0.0001***
Fibrinoid degeneration (number of villi)	0.000127	<0.001**
Hyperplasia of cytotrophoblast (number of villi)	0.0000	<0.0001***
Thick subtrophoblastic basement membrane (number of villi)	0.0000	<0.0001***
Avascular villi	0.02624	<0.05*

*Significant at 5% level of significance, **Significant at 0.1% level of significance, ***Significant at 0.01% level of significance

findings in passive smokers.[5] Spira *et al*. and Demir *et al*. had reported similar findings in the placentae of smokers.[6,7] Goujard *et al.*, Van der Veen and Fox, and Ashfaq *et al.* had stated that higher frequency of nuclear clumps in the perivillous syncytiotrophoblasts indicates hypoxia.[8-10]

As villi mature, syncytial knots develop on the villous surface and with time become more numerous, until at term, approximately 30% of the villi display knots[11] (27.07% ± 17.2% of the terminal villi in our control group displayed syncytial knots). An increase in the syncytial knots may be associated with an increase in the size of the knots and/or abnormal configurations. When these features are also present, the alteration is referred to as Tenney–Parker change. Increased syncytial knots are a sign of placental underperfusion. Since the smokers deciduas studied by Naeye had few of the arterial lesions that are characteristic of chronic low blood flow, he concluded that this underperfusion was probably periodic rather than continuous.[12] Nicotine via its acute effect of reducing intervillous placental blood flow could cause such a periodic uteroplacental underperfusion. On the contrary, Teasdale and Ghislaine found a significant reduction in syncytiotrophoblast nuclei in relative and absolute values in the placentae of smokers.[13] They attributed this reduction to lack of cytotrophoblastic cell proliferation and transformation into syncytiotrophoblasts as a fall-out of ischemia and/or toxic effects of tobacco chemical compounds on the placenta.

We observed an increase in fibrinoid degeneration of the terminal villi in our passive smokers (7.24% ± 8.01%), as compared to controls (3.36% ± 3.75%). Normal placentae show deposition of a modest amount of fibrinoid material in the intervillous space. Excessive fibrinoid deposition reduces intervillous blood flow, thereby compromising oxygenation of the fetus. Under a low power microscope, the net-like pattern can be identified, with areas of relatively normal villi adjacent to distinctly abnormal areas. Focally, the villi are encased in pink, hyaline deposits of fibrinoid. The villi initially appear viable, buried in their individual cloaks of fibrinoid. With time, the fibrinoid, being interposed between the maternal blood of the intervillous space and the trophoblast, literally chokes the villi, and they undergo degeneration and death. Kaufmann *et al*. stated that fibrin-type fibrinoid replaces degenerative syncytiotrophoblast at the maternofetal exchange surfaces, thus acting as a kind of substitute barrier.[14] Mayhew wrote that fibrinoid deposition during normal gestation is not only influenced by the quality of vascular perfusion but also emphasized that the villous surface is another important factor.[15] Hemostatic events operate at the maternal surface of trophoblastic epithelium and influence the steady state between coagulation and fibrinolysis. Fibrin is deposited at sites of trophoblast deepithelialization and these arise following trauma (e.g., abruption of intervillous bridges) or during the extrusion phase of normal epithelial turnover. Mayhew *et al*. observed that smoking during pregnancy perturbs maternal hemostasis via activated coagulation which could include greater coagulation (fibrin-type fibrinoid deposition) in the placental intervillous space.[16] Fibrin deposits could influence the sizes and numbers of intervillous spaces (pores), and perivillous fibrin could reflect changes in the nature or activity of trophoblast. In all placentae, the greatest deposits occurred, where there was trophoblast denudation.

In our study, cytotrophoblastic hyperplasia was observed in 29.97% ± 22.87% of terminal villi of passive smokers. The difference in comparison to the control group was statistically significant ($P < 0.0001$). Van der Veen and Fox found villous cytotrophoblastic cell proliferation in the placentae of smokers.[9] Rath *et al*. had reported cytotrophoblastic hyperplasia in 70.25% ± 14.7% of villi of passive smokers.[5] The increased percentage in their study was because they had counted the number of cells, while we have counted the number of villi. It could also be due to better appreciation of cytotrophoblasts in their 1-μ thick sections, as compared to our sections which were 3–5-μ thick. Castellucci *et al*., 1990 attributed cytotrophoblastic hyperplasia to failure of transformation of cytotrophoblast to syncytiotrophoblast mostly associated with placental hypoxia.[17]

The sub-TBM was thicker in 13.64% ± 13.17% of the terminal villi of passive smokers in our study in comparison to controls (4.88% ± 3.75%). Rath *et al*. noted appreciable thickening of basement membrane in passive smokers (75% of terminal villi studied).[5] Asmussen found marked thickening of TBM in smokers in his electron microscopic studies.[18,19] Van der Veen and Fox found an increase in TBM thickness in the placentae of smokers.[9] Ashfaq *et al*. reported a highly significant increase in sub-TBM thickness in wet snuff user placentae as compared to control.[10] According to Kaufmann *et al*., the increased thickness of TBM may be due to increased secretion of or decreased turnover of the basal lamina molecules.[14]

Marked thickening of the TBM is associated with various pathological conditions, such as preeclampsia, essential hypertension,[20] and maternal diabetes.[21,22] These confounding factors were carefully excluded by us. It is thought that many of these changes are as a result of placental ischemia, consequent upon nicotine-induced constriction of the uterine vessels; some of these changes cannot be explained on this basis. It is suggested that these may be possibly due to cadmium toxicity or to accumulation of polycyclic aromatic hydrocarbons.

The presence of irregular capillary lumina ($P = 0.0007$) and the absence of vasculosyncytial membrane in the terminal villi of passive smokers in comparison to control ($P = 0.0000$) have been reported in the same sample.[23]

There is probably some impairment of placental function in passive smokers; however, this is probably offset by the ability of the placenta to repair ischemic damage and to undergo a compensatory hyperplasia.

Conclusion

We conclude that maternal exposure to secondhand smoke could be associated with calcification on the fetal surface of the placenta. This is accompanied by histological changes, namely increased number of syncytial knots, increased cytotrophoblastic hyperplasia, fibrinoid degeneration, thickening of sub-TBM, and presence of more avascular villi. The accuracy of the study could be improved, by measuring serum cotinine levels in our cohort. However, most physicians rely on a well taken history to quantify exposure. The future scope for this study could be to correlate amount of exposure with extent of changes in the placenta at the gross and microscopic level. Correlating the placental changes observed with pregnancy outcome, birth-weight, and developmental milestones in the child could be future research in this field.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Luciano A, Bolognani M, Biondani P, Ghizzi C, Zoppi G, Signori E. The influence of maternal passive and light active smoking on intrauterine growth and body composition of the newborn. Eur J Clin Nutr 1998;52:760-3.
2. World Health Organization- Non-Communicable Diseases Country Profiles, WHO Document Production Services, Geneva, Switzerland; 2014:91.
3. Wenderlein JM. Smoking and pregnancy. Z Arztl Fortbild (Jena) 1995;89:467-71.
4. Christianson RE. Gross differences observed in the placentas of smokers and nonsmokers. Am J Epidemiol 1979;110:178-87.
5. Rath G, Jain AK, Bastia Sood M, Mukherjee A. The effects of passive smoking on the terminal villi of human placenta. J Anat Soc India 2001;50:19-23.
6. Spira A, Philippe E, Spira N, Dreyfus J, Schwartz D. Smoking during pregnancy and placental pathology. Biomedicine 1977;27:266-70.
7. Demir R, Demir AY, Yinanc M. Structural changes in placental barrier of smoking mother. A quantitative and ultrastructural study. Pathol Res Pract 1994;190:656-67.
8. Goujard J, Kaminski M, Rumeau-Rouquette C, Schwartz D. Maternal smoking, alcohol consumption, and abruptio placentae. Am J Obstet Gynecol 1978;130:738-9.
9. van der Veen F, Fox H. The effects of cigarette smoking on the human placenta: A light and electron microscopic study. Placenta 1982;3:243-56.
10. Ashfaq M, Channa MA, Malik MA, Khan D. Morphological changes in human placenta of wet snuff users. J Ayub Med Coll Abbottabad 2008;20:110-3.
11. Baerger RN, Benirschke K. The placenta, products of conception and gestational trophoblastic disease. Silverberg's Principles and Practice of Surgical Pathology and Cytopathology. 4th ed. Philadelphia, USA: Churchill Livingstone, Elsevier; 2006. p. 2071.
12. Naeye RL. Effects of maternal cigarette smoking on the fetus and placenta. Br J Obstet Gynaecol 1978;85:732-7.
13. Teasdale F, Ghislaine JJ. Morphological changes in the placentas of smoking mothers: A histomorphometric study. Biol Neonate 1989;55:251-9.
14. Kaufmann P, Huppertz B, Frank HG. The fibrinoids of the human placenta: Origin, composition and functional relevance. Ann Anat 1996;178:485-501.
15. Mayhew TM. Villous trophoblast of human placenta: A coherent view of its turnover, repair and contributions to villous development and maturation. Histol Histopathol 2001;16:1213-24.
16. Mayhew TM, Brotherton L, Holliday E, Orme G, Bush PG. Fibrin-type fibrinoid in placentae from pregnancies associated with maternal smoking: Association with villous trophoblast and impact on intervillous porosity. Placenta 2003;24:501-9.
17. Castellucci M, Kaufmann P, Bischof P. Extracellular matrix influences hormone and protein production by human chorionic villi. Cell Tissue Res 1990;262:135-42.
18. Asmussen I. Ultrastructure of the human placenta at term. Observations on placentas from newborn children of smoking and non-smoking mothers. Acta Obstet Gynecol Scand 1977;56:119-26.
19. Asmussen I. Ultrastructure of human umbilical veins. Observations on veins from newborn children of smoking and nonsmoking mothers. Acta Obstet Gynecol Scand 1978;57:253-5.
20. Fox H. Basement membrane changes in the villi of the human placenta. J Obstet Gynaecol Br Commonw 1968;75:302-6.
21. Liebhart M. Some observations on so-called fibrinoid necrosis of placental villi. An electron-microscopic study. Pathol Eur 1971;6:217-26.
22. Liebhart M. Polysaccharide surface coat (glycocalix) of human placental villi. Pathol Eur 1974;9:3-9.
23. Sreenivasan S, Khare S, Jain S, Khare A. The effects of passive smoking on the placental barrier: A light microscopic study. Peoples J Sci Res 2013;6:1-4.