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Research Article

EFFECTS OF PASSIVE SMOKING ON TERMINAL VILLI OF HUMAN PLACENTA AND GROWING FOETUS

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ABSTRACT

Background: Though ill effects of maternal smoking on growing foetus and placenta is well established, passive/ second hand smoking has recently been implicated as another potential risk factor. The incidence of low birth weight, Intra-Uterine Growth Retardation (IUGR), prematurity and stillbirth has been associated with passive smoking. Our study focuses on the gross and histological changes in the full term placenta of women exposed to passive smoke from a close member of a family smoking at least 20 cigarettes/bidis per day.
Method: The present study is an endeavour to observe microscopic features (light microscope under 10X and 40X only) of placenta of pregnant women who are exposed to environmental tobacco smoke from a close member of the family and simultaneously observe changes on growing foetus during level II ultrasound at 2nd trimester in terms of IUGR if any. This is a descriptive study with statistical analysis done using 'Independent Sample Test'.
Results: The histopathological changes seen in the placenta of passive smokers were almost alike to active smokers showing increased syncytial knots, hyperplasia of cytotrophoblast cells and proliferation of fetal capillaries. However no significant IUGR was noticed during Level II USG of the fetuses.
Conclusion: On gross findings there was a definite co-relation with low birth weight babies born to passive smokers and a compensatory enlargement of the placenta. The microscopic changes seen in the placenta of passive smokers was a consistent finding similar to ischemic changes. No definite relationship could be established between the histological changes observed amongst passive smokers and IUGR seen in the growing foetus.

INTRODUCTION

The Human reproduction process from fertilisation to birth is full with difficulties that lower fertility. The inherent problems are enhanced when women smoke and lead to adverse pregnancy outcomes.[1] Once blastocyst is implanted, smoking substantially increases a woman's risk of a number of serious complications including abruption, preterm delivery and low birth weight babies that are strongly associated with infant morbidity and mortality.[2]

Nicotine a major component of smoking tobacco (cigarettes or bidis) has been implicated in producing toxic effects on developing foetus and placenta. [3,4]. Though ill effects of maternal smoking on growing foetus and placenta is well established, passive/ second hand smoking or Environmental Tobacco Smoke(ETS) exposure has recently been focused on as another potential risk factor.

When a pregnant lady is exposed to second hand smoking she breathes environmental oxygen partially replaced by carbon monoxide. Carbon monoxide binds preferentially to foetal haemoglobin. The higher level of carboxyhaemoglobin suppresses oxygen supply to foetal tissues thus leading to hypoxia in the foetus.[5] Nicotine moves directly into the lining of small blood vessels causing them to constrict, thereby reducing blood flow to the growing foetus. Nicotine readily crosses the placenta and can show 15% higher levels than mother.[6] Prostacyclins which are potent vasodilators are also inhibited by higher levels of nicotine. Thus vascular resistance of uterus is enhanced leading to reduced blood flow to the organ. A

likely cause of foetal growth retardation is induction of foetal ischemia and hypoxia as a result of effect of nicotine on the placental circulation.[7]

Studies on the influence of smoking on gross and microscopic features of placental have started to explain the pathophysiological mechanisms involved. Microscopic changes including cytotrophoblast hyperplasia, thickening of trophoblast basement membrane, and decreased capillary density at the terminal villi are believed to contribute to decreased placental oxygen and nutrient transfer.[8]

Paffer et al noticed correlation of increased placental weight with corresponding decrease in foetal weight in mothers with history of smoking tobacco during pregnancy.[9] Demir et al noticed increased syncytial knots in the placenta of active smokers.[10] The increase in syncitial knots were attributed to degenerative changes due to ischemic conditions by Vander Veen and Fox.[11]

The present study is an endeavour to observe microscopic features (light microscope only) of placenta of pregnant women who are exposed to environmental tobacco smoke from a close member of the family smoking more than 20 cigarettes / bidis a day and simultaneously observe USG changes on foetus at 2nd or 3rd trimester in terms of IUGR if any.

MATERIALS AND METHODS

The study was carried out over a period of 2 years in collaboration with Dept of Anatomy, Armed Forces Medical College and Dept of Obstetrics and Gynaecology, Command Hospital, Pune. Necessary clearance was obtained from Institute Ethical Committee, AFMC. About one hundred placentas were collected from labour room soon after delivery after taking consent from the mothers. Out of hundred placentas fifty were from women without history of Passive smoking and served as control. The other fifty placentas were collected from women with history of passive smoking. The passive smokers in this study were defined as pregnant ladies with husband or any other close relative residing in the same house smoking 20- 30 cigarettes/ bidis per day. Sampling method was simple random sampling.

Only those placentas that suited our study were collected. The exclusion criteria being antenatal history of pregnancy induced hypertension, gestational diabetes, pre-eclampsia/ eclampsia, abruptio placentae and known cases of IDDM/NIDDM, primary hypertension, alcohol and tobacco abuse. They were weighed in the labour room and exact weight was noted down in grams after removing the membranes and clots. Each of these placentas was fixed in 10% Formalin for 24hrs before they were taken for Grossing. From the maternal surface two samples were taken, one from centre and the other from periphery of about 1cm in thickness.

The tissues were embedded in paraffin blocks and slides were made of 3-5µ thickness. Each slide was stained using Haematoxylin and Eosin (H&E) stain and seen under light microscope. As many as 100 terminal villi were microscopically examined for syncytial knots, degenerative changes in syncytiotrophoblast, no. of cytotrophoblast cells and endothelial cell protrusion in foetal capillaries. The photographs were taken using high resolution digital camera.

The study was restricted to histopathological changes seen in terminal villi. The quality of tobacco was not taken into consideration. The quantity of nicotine in blood of pregnant mother was also not estimated.

The routine Level II Utrasound report of growing foetus at second trimester was collected from Antenatal card/ radiology dept/ gynaecology OPD. The USG report was looked at for evidence of IUGR and compensatory increase of placental size if any.

A "Descriptive" statistical study was carried out calculating the mean and standard deviation of the collected values of both gross and histopathological findings. The positive radiological finding in terms of IUGR was also noted down in the cases (no IUGR was seen in control group).

Thereafter "Analytic" statistical study was carried out using "Independent Samples Test". The t value was looked for both by equal variances assumed and equal variances not assumed. The P value was derived for level of significance.

RESULTS

In our study abnormality in gross anatomical features were minimally noticed. There was not much difference in terms of shape and size between placentas of control and passive smoker groups.

The average weight of the placenta of passive smoker group was found to be more when compared with control group. On an average the cases showed an 80gms increase in weight of placenta as compared to the control. There was also compensatory loss of weight of the delivered infant. The infants of the case group showed birth weight that was roughly 400gms lighter than the infants of control group (Table 1).

The fixed placental tissue stained in H&E was seen under 10X and 40X objective lens of Olympus bright field microscope. Each field showed numerous cut sections of term villi with intervillous space filled with maternal blood. The following parameters were used to compare microscopic changes in the placental tissue of control group and passive smokers:

1. Number of Syncytial knots/buds per 100villi
2. Degree of necrosis and degenerative changes seen in Syncytiotrophblast and the number of such nuclei per 100villi
3. No. of cytotrophoblst cells present in a full term placenta per 100 villi
4. No. of endothelial cells that protrude in the lumen of the capillaries per 100 villi

The histological data collected is tabulated as a comparison between control and cases (Table 2). All microscopic features tabulated are under 40X objective and 10X eyepiece of bright field microscope.

Syncytiotrophoblast:

The Syncytiotrophoblast which is the darker staining outer layer consisting of multiple nuclei showed varied degree of necrosis and degenerative changes. These nuclei in passive smokers showed high irregularity with fragmented chromatin material. At places the nuclei grouped together to protrude in the intervillous spaces forming Syncytial knots or buds (Fig 1). Though Syncytial knots were a common finding with both the cases and control groups, their numbers were definitely more amongst passive smokers.

Cytotrophoblast:

The Cytotrophoblast which forms the inner lighter staining layer almost disappears at full term. However in our cases of passive smokers presence of these cells were a

consistent feature. The presence of 60 to 65 such cells per 100 villi was indicative of cytotrophoblast hyperplasia. The thickness and character of its basement membrane could not be commented upon at 40x magnification with H&E stain.

Capillaries:

At term the fetal capillaries increase in number and abut closely to syncytiotrophoblast. The capillaries are lined by endothelium giving the lumen a smooth appearance. However in the passive smokers the lumen appeared irregular with number of endothelial cells jutting into the lumen. Almost about 40 such cells were seen per 100 villi in passive smokers as compared to just 7-8 such cells in control group (Fig 2).

Ultrasonography:

The patients of both the control group and passive smokers underwent routine Level II Ultrasonography at second trimester. In my study there was no evidence of IUGR amongst the passive smokers (Fig 3).

Table 1: Group statistics- Placental & Infant weights

S.No.	Parameters Groups	N	Mean	Standard Deviation	Std. Error Mean
1.	Infant weight Cases (kgs) Control	50 50	2.398 2.798	0.228 0.164	3.223E-02 2.325E-02
2.	Placental weight Cases (gms) Control	50 50	525.66 444.16	25.94 31.31	3.67 4.43

Table 2: Histological findings in placenta of control and cases

S.No.	Histological findings	Control (50)	Passive Smokers(50)
1.	Syncytial knots	21.66 ± 6.56	41.40 ± 11.10
2.	Syncytiotrophoblast	11.06 ± 2.96	37.88 ± 4.92
3.	Cytotrophoblast	6.92 ± 2.40	64.86 ± 14.41
4.	Endothelial cell protrusion	8.76 ± 3.12	38.54 ± 9.15

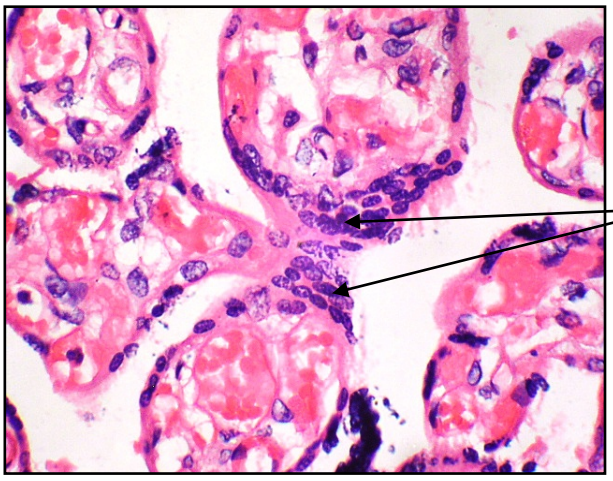

Fig1: Syncytial knots seen in histology of terminal villi of placenta of passive smokers (under 40X objective lens)

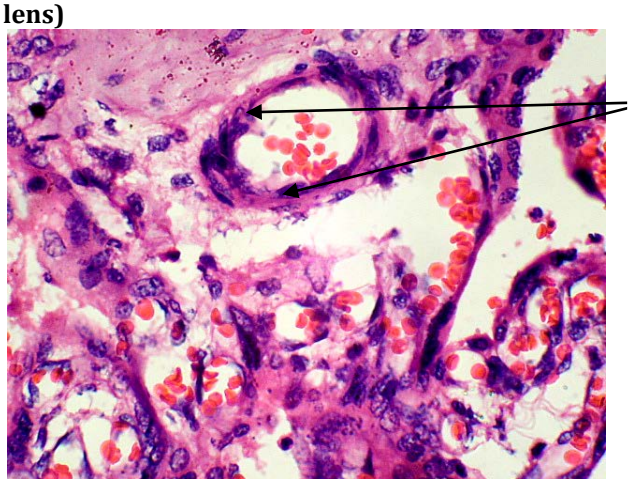

Fig 2: Endothelium cell protrusion seen in histology of terminal villi of placenta of passive smokers (under 40X objective lens)

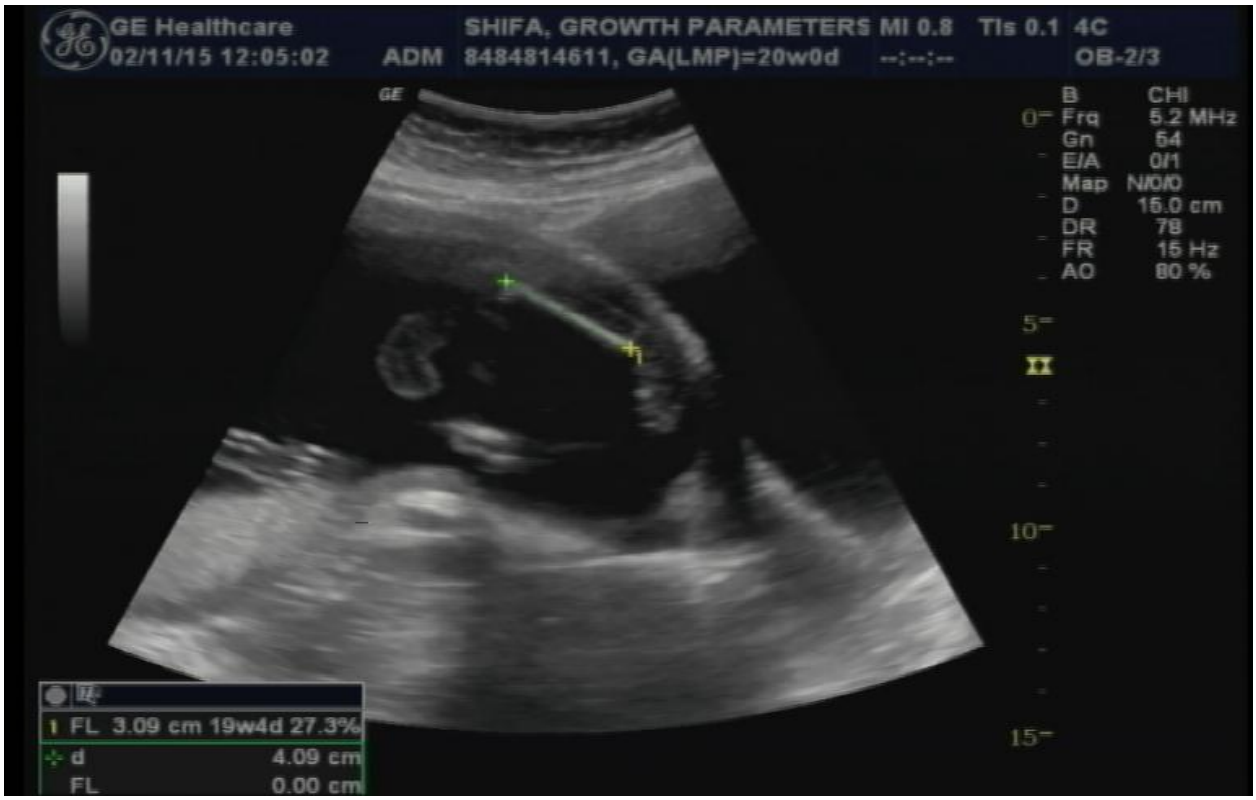

Fig 3 (a): Level II USG at 20 weeks in a passive smoker showing normal intra-uterine growth (Femoral length 3.09 cms equivalent to 19 wks 04 days)

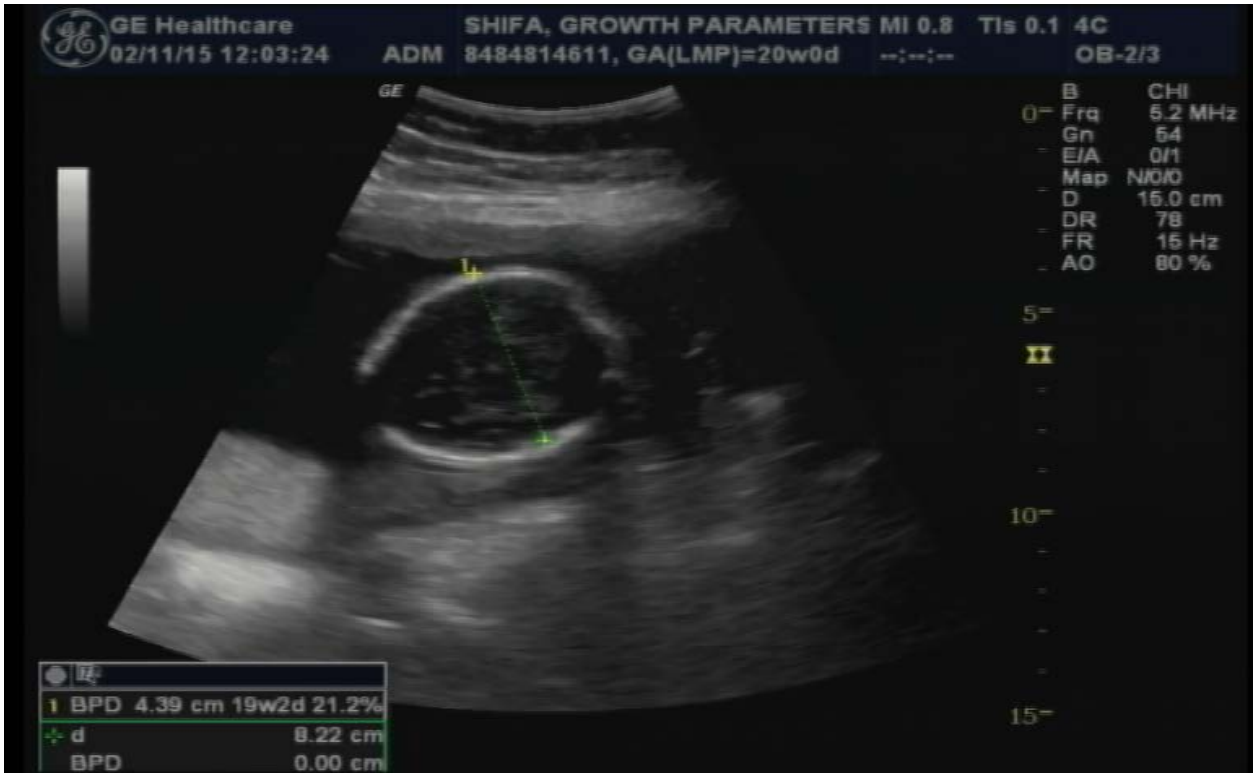

Fig 3 (b): Level II USG at 20 weeks in the passive smoker showing normal intra-uterine growth (Biparietal diameter 4.39 cms equivalent to 19 wks 02 days)

DISCUSSION

Maternal smoking has been associated with marked ultra structural and functional changes in the placenta. Women whose partner smoked 20 or more cigarettes a day staying together also showed similar changes in placenta. Such changes including cytotrophoblast hyperplasia, thickened trophoblastic membrane and decreased capillary density at terminal villi are believed to contribute to reduced placental nutrient and oxygen transfer leading to adverse pregnancy outcomes.[12]

Hruba D & Kachlik P reported that the newly born infants of smoking mothers presented twice the risk of being born underweight and adverse consequences as a result of it.[13] Mathai et al studied a South Indian population group for correlation between low birth weight babies and passive smokers and concluded a strong association between them.[14] In our study similar effect was noted. The mean weight of babies born to passive smokers was almost 400gms less than control group. It was also noticed that there was a compensatory increase in average weight of placenta by 80gms in the passive smokers.

The histopathological changes seen in the placenta of passive smokers are almost alike to active smokers.[15] Demir et al in their study noticed increased syncytial knots in the syncytiotrophoblast layer of the placenta of active smokers.[10] According to Vander Veen and Fox, nicotine and carbon monoxide induces hypoxia and ischemia leading to these degenerative changes in the syncytiotrophoblast.[11]In our study 41.4 % of the terminal villi of placenta of passive smokers showed presence of syncytial knots as compared with 21.7% in controlled group.

Andree Gruslin et al in their study of influence of maternal smoking on trophoblast apoptosis stated that apoptosis which is a physiological form of cell death increases at end of gestation in normal mothers while in smokers/passive smokers placental apoptosis decreased at term.[16] This apoptosis was seen to be inhibited in the syncytiotrophoblast layer also.[17] Thus on one hand there is increased degenerative changes seen in the syncytiotrophoblast layer and on the other hand there is cytotrophoblastic hyperplasia with poor differentiation in the cytotrophoblastic layer.[18] In our study also cytotrophoblastic hyperplasia was seen in passive smokers with as many as 64.86 cytotrophoblasts seen on average per 100 villi compared to just 6.92% in control group.

Habashi et al (1983) and Asmussen (1980) suggested that there was marked decrease in number of fetal capillaries in the terminal villi of smoking mothers.[19] They also suggested that the lumen of the capillaries were constricted and surrounded by increased amount of collagen deposit which was perhaps the cause of reduced blood flow to the growing fetus and subsequent growth retardation. In our study however mild proliferation of the capillaries were seen in the passive smokers as compared to the control group. This could be attributed to angiogenesis in response to ischemia.

The routine level II USG was performed at 2nd trimester around 20 weeks in both the control group and passive smokers and was specifically looked for growth parameters in terms of femoral length and biparietal diameter. In our study we could not establish statistically significant data to attribute definite co-relation between IUGR and passive smoking.

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