EFFECTS OF MATERNAL SMOKING ON PLACENTAL MORPHOLOGY

Muhammad Ashfaq, Muhammad Zahoor Janjua*, Muhammad Nawaz
Department of Anatomy, Ayub Medical College, Abbottabad and *Basic Medical Science Institute, JPMC, Karachi.

Back ground: Maternal smoking is one of leading causes of premature labour and low birth weight babies. Nicotine and carbon monoxide both induce degenerative changes as well as premature aging of placenta. Degenerative changes induce increased amount of collagen in chorionic villi and increased thickness of subtrophoblastic basement membrane. Premature aging is indicated by increased number of syncytial buds and higher percentage of apoptosis in smoker's placentae. Premature aging and degenerative changes may reduce the functional component of placenta and lead to abnormal outcome of pregnancy. This study was designed to determine the effects of maternal smoking on placental morphology. **Methods:** Total 40 full term placentae, 20 from normal and 20 from smoker mothers were studied histologically. Full thickness pieces of each placenta from standard area were taken for paraffin embedment. Four micron thick sections were cut on rotary microtome and stained with haematoxylin and Eosin, Malloryis trichrome and hexamine silver for syncytial buds, Apoptotic cells, chorionic villous collagen and Subtrophoblastic basement membrane. **Results:** This study demonstrates that there is extensive aging and degenerative changes in smoker's placentae. The aging process is shown by increased syncytial buds per unit area and high percentage of apoptosis. Degenerative changes are indicated by increased amount of collagen in chorionic villi and increased thickness of subtrophoblastic basement membrane. **Conclusion:** Extensive premature aging and degenerative changes in smoker's placentae decrease the functional component of an organ, reducing its nutritive and excretory functions. This may be the cause of low birth weight babies in smokers. Extensive loss of trophoblasts by apoptosis and syncytial buds may lead to hormonal imbalance and premature labour in smokers.

Keywords: Placenta, Smoking, Degeneration, Aging

INTRODUCTION

Placenta functions as respiratory, excretory and nutritive organ between foetus and mother. It also produces various hormones, which are necessary for continuation of normal pregnancy. The exchange of materials, between foetus and mother takes place at feto-maternal membrane which separates maternal blood in the intervillous space from foetal circulation[1]. The feto-placental membrane is composed of foetal vascular endothelial cells and their basement membranes, connective tissue of the villous, the subepithelial basement membrane and its covering of cyto and syncytio trophoblasts[2]. This barrier allows water, oxygen, other nutritive substances and hormones to pass from mother to foetus, and some of the products of excretion from foetus to mother. Functionally, this feto-maternal membrane is most important part of placenta.

Use of tobacco has increased in recent years, especially in adolescent and adults. Out of 3,000 active ingredients in smoke, nicotine is the most dangerous and can affect the placental tissue[3]. It also affects the foetus directly as it crosses placental barrier easily due to its high fat solubility[4]. Due to accentuation of degenerative and premature aging processes in placenta by addiction to tobacco, transfer of substance across feto-maternal membrane is badly affected which may lead to deleterious effects on out come of pregnancy.

MATERIAL AND METHODS

In this study 40 term placentae from normal and smoker subjects were used. These placentae were collected during 6 months period. The samples obtained from the Department of Obstetrics and Gynaecology, Unit-I, Jinnah Postgraduate Medical Centre, Karachi. The placentae were preserved in 10% formalin for at least five days before the sections from these placentae were taken for histological observations.

All subjects included in this study were healthy looking multiparous mothers aged between 25–35 years. There were no racial, cultural or environmental differences among the subjects. Heights and weights of all the subjects were comparable.

A total of 40 placentae, all of male babies, divided into two groups, were selected for the study. Those subjects suffering from obstetric abnormalities, i.e., abruptio placentae, twins, and jaundice were excluded from this study. All placentae obtained were of normal vaginal deliveries.

Group A:

In this group those 20 placentae from pregnancies, which were not complicated by any disease or mother's addiction to any substance, were included.

Group B:

Twenty placentae from moderate smoker mothers (smoking less than 10 cigarettes per day) were included in this group. Placentae in both groups were microscopically studied for degenerative and aging processes.

a. Degenerative changes:

i. Amount of chorionic villous collagen.
ii. Subtrophoblastic basement membrane thickness.

b. Aging process:

i. Percentage of apoptotic cells.

ii. Average number of syncytial buds per unit area.

TISSUE PROCESSING FOR SECTIONING:

Paraffin Sections:

Placentae fixed in 10% formalin were processed for routine paraffin embedment. Tissue pieces measuring 2×2 Cm from standard area, i.e., 2 Cm from the edge and 2 Cm from the attachment of umbilical cord were taken. 4 micron thick sections were cut on rotary microtome from the middle of each specimen, and were mounted on clean gelatinized slides, stained with H & E, Mallory's trichrome and hexamine silver.

Haematoxylin and Eosin:

This stain was used to study the percentage of apoptotic cells and average number of syncytial buds per unit area. These cells and buds were counted with the help of reticule on light microscope at ×40 objective and ×8 occular.

Mallory's Trichrome:

This stain was used to study the collagen in the cores of chorionic villi. collagen takes blue colour with Mallory's trichrome. Total number of chorionic villi per unit area, showing excessive amount of collagen was counted with the help of reticule at five random fields for all placentae included in this study. Their average was then calculated for group A & B.

Hexamine Silver:

This stain was used to study the thickness of subtrophoblastic basement membrane. The basement membrane took black colour as the silver in this stain was reduced by aldehydes in the basement membrane. Thickness of basement membrane was measured with the help of ocular micrometer.

Statistical analysis:

The statistical significance of the difference between two means of various parameters between two groups was evaluated by Student's 't-test'[4]. The difference was regarded as highly significant if *p* value was less than 0.001, statistically significant if *p* value was less than 0.05, and non significant if *p* value was greater than 0.05.

RESULTS

Group A: (Full term normal placentae)

(a) Degenerative changes:

i. Amount of chorionic villous collagen: Average number of villi showing excessive collagen was found. 4.62±0.32/0.0576 mm^2 (Table-1).

ii. Thickness of the subtrophoblastic basement membrane in group A was not measurable with the help of ocular micrometer as the lowest measuring unit on ocular micrometer was calibrated 1.1 µm on ×100, while thickness of basement membrane was less than 1.1 µm.

(b) Aging Process:

i. Percentage of apoptosis was 0.45±0.07 % (Table-2).

ii. Average number of syncytial buds per 0.0576 mm^2 was 5.92±0.30 (Table-3).

Group B: (Full term smokers' placentae)

(a) Degenerative Changes:

i. Average number of villi showing excessive amount of collagen was 11.88±0.65/0.0576 mm^2 (Table-1).

(a) The average thickness of subtrophoblastic basement membrane was calculated and found 1.2 µm.

(b) Aging Process;

i. Percentage of apoptosis counted was 1.06±0.05 % (Table-2).

ii. Average number of syncytial buds per unit area was found 16.10±0.91/0.0576 mm^2 (Table-3).

Table-1: Number of chorionic villi/0.0516 mm^2 with excessive collagen.

Parameter	Group A	Group B
Chorionic villi /0.0516 mm^2	4.62±32	11.88± 0.65

p <0.001

Table-2: Percentage of apoptosis

Parameter	Group A	Group B
Percentage of Apoptosis	0.45 %±0.07	1.06 %±0.05

p <0.001

Table-3: No of syncytial knots/0.0516 mm^2

Parameter	Group A	Group B
Syncytial Knots/0.0576 mm^2	5.92± 0.30	16.10± 0.91

p <0.001

Statistical analysis shows highly significant increase in average number of chorionic villi with excessive collagen, syncytial buds and apoptotic cells per unit area in group B when compared with group A.

DISCUSSION

Developmental changes in normal placentae during nine months of its intrauterine existence are considered as an aging process in an organ with short life span[14]. In smokers placentae morphological changes consist, in large part, of an intensification of degenerative and aging process observed in normal placenta. These changes include increased stromal fibrosis, excessive thickening of subtrophoblastic basement membrane, enlarged and more syncytial buds per unit area, and very high incidence of apoptosis in parenchymal cells of placenta. These findings are similar to those reported by Kerr *et al*[15] who reported atrophy and involution of tissues and organs undergoing extensive apoptosis. They also reported increased apoptosis in tissues facing ischemia. The results are also in accordance with those reported by Tominaga who found extensive syncytial bud formation in hypoxic conditions of placenta[13].

Cigarette smoke contains about 3000 active ingredients but nicotine and carbon monoxide have obstetrical importance[5]. Nicotine triggers rapid elevation in maternal plasma concentration of catecholamine resulting increased maternal blood pressure.

It also reduces foetal and maternal vascular prostaglandin I_2 (PGI_2). Particularly in placenta and umbilical card through cyclo-oxygenase inhibition[6]. Increased maternal blood pressure and inhibition of PGI_2 by nicotine may lead to reduced uterojdacental blood flow. In this way chorionic villi suffer from hypoxia which affects the parenchyma of an organ. Carbon monoxide in cigarette smoke produces functional anaemia in smoke because of its higher affinity for haemoglobin than oxygen[7]. This process further aggravates the hypoxia already produced by vasoconstrictive effects of nicotine on uteroplacental vessels. In response to this hypoxia above-mentioned changes have been noted in parenchyma of smoker's placenta.

Hypoxia, nicotine and carbon monoxide are stimuli for increased apoptosis in smoker's placenta[8]. Due to increased incidence of apoptosis, large number of parenchyma cells have been observed to be eliminated and replaced by fibrous tissue[9]. This fibrous tissue was synthesized by fibroblasts of villous stroma[10]. Fibroblasts also take part in the synthesis of subtrophodastic basements membrane[11]. In this way villous collagen increases in cigarette smokers and increased collagen in villi affects the subtrophoblastic basement membrane leading to its increased thickening.

Smokers placenta have shown a highly significant increase in number and size of syncytial buds when compared with normal placenta. This morphological response to hypoxia in cigarette smoker's represents a useful accommodation. Its apparent purpose would be to reduce the distance which oxygen must travel between maternal and foetal plasma but increased loss of trophoblasts in the form of these buds may lead to hormonal imbalance and premature labour[12]. Increased villous collagen and increased thickening of subtrophoblastic basement membrane may lead to increased thickening of placentae barrier between foetal and maternal blood and this may in turn reduce the exchange of materials across placenta[13]. This may be the cause of low birth weight babies in smoker mothers.

Figure-1: Photomicrograph of a 4 µ thick H & E stained paraffin section of full term normal human placenta from group A showing less prominent and scanty syncytial buds against the arrows heads. ×416.

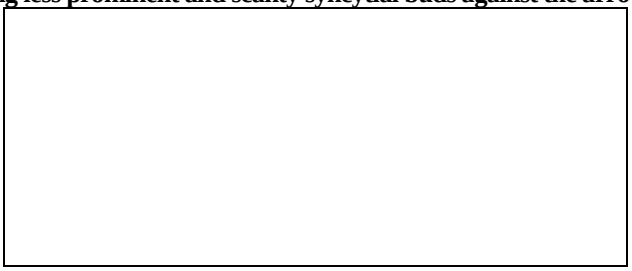


Figure-2: Photomicrograph of a 4 µ thick H & E stained paraffin section of full term smoker's human placenta from group B showing prominent and numerous syncytial buds against the arrow heads. ×416.

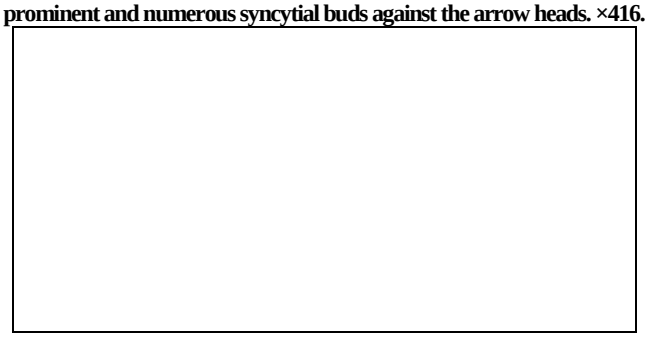


Figure-3: Photomicrograph of a 4 µ thick Mallory's trichrome stained paraffin section of full term human placenta from group A showing scanty chorionic villous collagen. ×416.

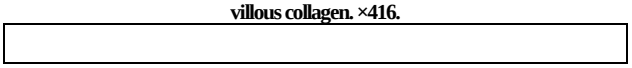
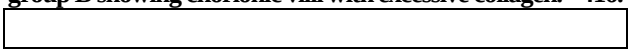


Figure-4: Photomicrograph of a 4 µ thick Mallory's trichrome stained paraffin section of full term human placenta from group B showing chorionic villi with excessive collagen. ×416.



On the basis of results of present study, it is concluded that the normal aging process in placenta which is adaptive response to increasing workload as the pregnancy advances, can turn into pathological change when exposed to abnormal environment and chemicals like nicotine, and carbon monoxide. This pathological change in placenta can result in abnormal outcome of pregnancy if placenta remains exposed to such stimuli.

ACKNOWLEDGEMENT

The authors wish to thank the staff of Anatomy Department BMSI and Gynaecology & Obstetrics Unit JPMC Karachi for their cooperation.

REFERENCE

1. Moore KL. The developing human, 3rd ed, Philadelphia; WB Saunders 1983; PP 65-6.
2. Guyton AC. Text book of medical physiology 8th ed, Philadelphia; WB Saunders, 1991; PP 917-9.
3. Katzung BG. Basic and clinical pharmacology, 7th ed., San Francisco, Appleton and Lange, 1998; PP 90-2.
4. Hill AB. A short text book of medical statistics, 10th ed, London; ELBS, 1977;PP 127-36.
5. Lehtovirta P, Fross M, Kariniemi V, Ruuramo I. Acute effects of smoking on foetal heart rate variability. Br J Obstet Gynaecology 1983; 90:3-6.
6. Dadak CH, Leithner CH, Sinzinger H, Silberbauer K. Diminished prostaglandin formation in umbilical arteries of babies born to women who smoke. Lancet 1981;1:94-5.
7. Wingerd J, Christianson R, Lovitt WV, Schoen EJ. Placental ration in white and black women. Ratio to smoking and anaemia. Am J Obstet Gynaecol 1976;124:671-90.
8. Smith SC, Baker PN, Symonds ME. Placental apoptosis in normal human pregnancy. Am J Obstet Gynecol 1997;177:57-65.
9. Majno G, Joris I. Cells, Tissues and disease, Principle of general pathology, 2nd ed, Massachusetts, Blackwell Science, 1996;PP 681-2.
10. Mallory FB, Parker F. Reticulum. Am J Path 1927;3:515-7.
11. Tenny B Jr. Study of collagen of placenta. Am J Obstet Gynecol 1934;29:819-25.
12. Mulcahy R, Murphy J, Martin F. Placental changes and maternal weight in smoking and non smoking mothers. Am J Obstet Gynecol 1970;106:703-4.
13. Tominaga T, Page EW. Accommodation of human placenta in hypoxia. Am J Obstet Gynecol 1966;94:679-91.
14. Bartholomew RAa, Kracke RR. Histogenesis of degenerative process in normal mature placenta. Am J Obstet Gynecol 1932;24:797-801.
15. Kerr JFR, Wylie AH, Cume AR. Apoptosis: a basic biological phenomenon with wide ranging implications in tissue kinetics. Br J Cancer 1972;26:239-57.

Address for correspondence:
Dr. Muhammad Ashfaq, Assistant Professor in Anatomy, Ayub Medical College, Abbottabad-22040, Pakistan. Tele: +92 992 381907 /3049.