MORPHOLOGICAL CHANGES IN HUMAN PLACENTA OF WET SNUFF USERS

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Background: Wet snuff is commonly used by both males and females in different parts of Pakistan. Apart from other ingredients, tobacco is the major component of snuff. Adverse effects of smoking on morphology of human placenta have been shown by some previous studies. But snuff is not considered as dangerous as smoking during pregnancy. This study was designed to see the effects of snuff on morphology of human placenta. **Methods:** In present study total 80 human placentae, 40 from normal and 40 from snuff users were used. This study was carried out in the Department of Anatomy Basic Medical Sciences Institution (BSMI) Karachi. Duration of study was six months. Samples were obtained from Gynaecology and Obstetric unit-I JPMC. Placentae washed well with running tap water to remove blood clots. Umbilical cord and other membranes were removed and placenta gently squeezed to expel the foetal blood. Gross features like weight, diameters, central thickness and attachment of umbilical cord were noted in normal and snuff users' placentae. Then placentae were preserved in 10% formalin for at least five days before the sectioning for micromorphology. Placentae divided in two groups-A & B. 4 µm thick sections of the tissue were taken on rotary microtome and stained with H & E, Mallorys trichrome and methanamine silver for different histological observations. **Results:** Mircromorpholgical changes have been observed in placentae of snuff users leading to loss of functional components of placentae. This loss of functional component may have deleterious effects on outcome of pregnancy. No significant gross morphological changes were found in snuff user placentae. **Conclusion:** Wet snuff effect the micro-morphology of placenta leading to loss of functional component and in turn effects the exchange of materials between mother and foetus which may leads to intrauterine growth retardation. Loss of trophoblasts may lead to hormonal imbalance necessary for normal pregnancy and this imbalance can cause premature labour. Nocotine can cross the placental barrier[2], which may produce foetal tachycardia.

Keywords: human placenta, snuff, nicotine.

INTRODUCTION

Wet snuff is commonly used by both males and females in different parts of Pakistan. Apart from other ingredients, tobacco is the major component of snuff. Adverse effects of smoking on morphology of human placenta have been shown by some previous studies.[1] Placenta functions as respiratory, excretory and nutritive organ between foetus and mother. It also produces various hormones, which are necessary for continuation of pregnancy.[3] The exchange of materials between foetus and mother takes place at foeto-maternal barrier, which separates maternal blood in the inter-villous space from foetal circulation[4] This foeto-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[4] This barrier allows water oxygen other nutritive substances and hormones to pass from mother to foetus and some of the products of exertion from foetus to mother. Placental hormones are secreted by trphoplasts.[5]

Functionally this foeto-maternal barrier is most important part of placenta. Maternal use of wet snuff during pregnancy can affect the tissue components of placenta due to tobacco used in snuff preparation.[6] Nicotine is most dangerous component of tobacco, which can affect placental tissue directly or by indirect effect produced by vasoconstriction of maternal vessels[7]

Due to accentuation of degenerative and aging changes in placenta by use of snuff, transfer of substances across feoto-maternal barrier is badly affected which can lead to deleterious effects on the outcome of pregnancy. This study is undertaken to determine the effects of snuff on morphology of placenta and to confirm the magnitude of damage caused by snuff to human placenta.

MATERIAL & METHODS

In present study total 80 term placentae 40 from normal and 40 from snuff users were used. Samples obtained from Department of obstetrics and Gynaecology Unit-I JPMC Karachi. The placentae were obtained in 10% formaline.

Placentae were washed well with running tap water to remove the blood clots from maternal surface of placenta. Unbiblical cords were cut 1cm above their attachments on the foetal surface and foetal vessels were gently squeezed to expel the blood via umbilical vessels. Other membranes were removed from placentae.

Gross morphological features, weight, diameter central thickness and attachment of umbilical cord were noted in normal and snuff users' placenta.

The placentae were then preserved in 10% formaline for at least five days before the sectioning for histological observations.

Prior to collection of samples from labour room, the subjects were examined in the ward and brief histories related to use of snuff were taken. All

the subjects included in this study were healthy looking multiperous mothers aged between 25 to 35 years. Heights and weights of subjects were comparable. There were no racial, cultural or environmental difference among the subjects. Total number of 80 placentae divided in two groups A&B were selected for the study. Those subjects suffering from obstetric abnormalities, i.e., abruptio placenta, twins, congestive cardiac failure, jaundice, hypertension, diabetes and smoking were excluded from this study. All placentae obtained were of normal vaginal deliveries.

Group-A (Normal full term placentae)
In this group 40 placentae from pregnancies, which were not complicated by any disease or mother's addiction to any substance were included.

Group-B (Full term snuff users' plancetae)
Forty placentae from snuff users for more than two years were included in this group.
Placentae from each group A & B were studied morphologically.

The micromorphologic features included this study were

a. Degenerative changes
   1. Amount of chorionic villous collagen
   2. Trophoblastic basement membrane thickness.
b. Aging process in placenta
   1. Percentage of apoptotic cells
   2. Average number of syncytial knots per unit area.

TISSUE PROCESSING FOR SECTIONING

Paraffin sections

Placentae fixed in 10% formaline were processed for routine paraffin embedment. Tissue pieces measuring 2x2cm from standard area, i.e. 2cm from edge and 2cm from the attachment of umbilical cord were taken. Four-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 methanamine sliver.

H & E was used to study the percentage of apoptotic cells and average number of syncytial knots per unit area in groups A & B. these cells and buds were counted with the help of reticule.

Mallory's trichrome was used to study the amount of collagen in the cores of chorionic villi in group A and B. Total number of chorionic vill per unit area, showing the excessive amount of collagen at 40x objective and 8x ocular with the help of reticule at six random fields were calculated from all placentae in this study. Then the average was calculated for group A & B.

Sliver methanamine was used to study the thickness of trophoplastic basement membrane in placenta of two groups included in this study. Basement membrane took black colour with this stain. Thickness of basement membrane was measured with help of ocular micrometer.

The statistical significance of the difference between two means of various parameters was evaluated by Student's *t*-test.

RESULTS

Micromorphology

Group-A (Full term normal placentae)

a. Degenerative changes
   i. Amount of chorionic villous collagen. Average number of chorionic villi showing excessive collagen was found 4.62±0.32/0.0576 mm^2 (Table-1).
   ii. Thickness of the subtrophoplastic basement membrane in group-A was not measurable with the help of ocular micrometer as the lowest measuring unit on occular 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-1)
   ii. Average number of syncytial buds per 0.0576 mm^2 was 5.92±0.30 (Table-1)

Group-B

a. Degenerative changes
   i. Average number of villi showing excessive amount of collagen was 9.32±0.45/0.0576 mm^2 (Table-1)
   ii. The average thickness of subtrophorblastic basement membrane was calculated and found 2 μm (Table-1)
b. Aging process
   i. Percentage of apoptosis counted was 1.20±0.07% (Table-1).
   ii. Average number of syncytial buds per unit area was found 12.20±0.95/0.0576 mm^2 (Table-1).

Statistical analysis shows highly significant increase in average number of chorionic villi with excessive collagen, syncytial buds & apoptotic cells per unit area in group B when compared with Group A Subtrophoblastic basement membrane thickness is also highly significantly increased in group B.

Table-1: Comparison of microscopic changes in placenta among wet snuff and non-wet snuff users

Parameter	Group A	Group B	*p*-Value
Chorionic villi with excessive collagen	4.62±0.32	9.32±0.45	<0.001
Percentage of apoptosis	0.45±0.07	1.20±0.07%	<0.001
Syncytial knots/0.0576 mm^2	5.92±0.30	12.20±0.95	<0.001
Subtrophoblastic basement membrane	Less than 1.1 μm	2.00 μm	>0.001

Gross Morphology

Group A (Full term normal placentae)

The mean weight of placenta in each group is given in Table-2. The difference between group A & B was statistically non-significant ($p>0.05$)

Mean diameter of placenta in both groups is given in Table-2. There is non-significant difference in diameter between normal and snuff user's placentae ($p>0.05$)

Central thickness of all placentae in group A & B was measured in centimetres. The mean central thickness of group B, when compared with group A (control), non-significant statistical difference was found ($p>0.05$) comparison is shown in Table-2.

In all placentae from both groups A and B attachment of umbilical cord was found central on the foetal surface of placenta.

No abnormality of cord attachment was found in snuff user placentae.

Table-2: Comparison of gross changes in placenta among wet snuff users and nonusers

Parameter	Group A	Group B	*p-Value*
Weight (gm)	53±10	474±11.27	>0.05
Diameter (cm)	14.26±00.19	13.44±0.014	>0.05
Central thickness (cm)	2.15±00.16	2.10± 00.02	>0.05

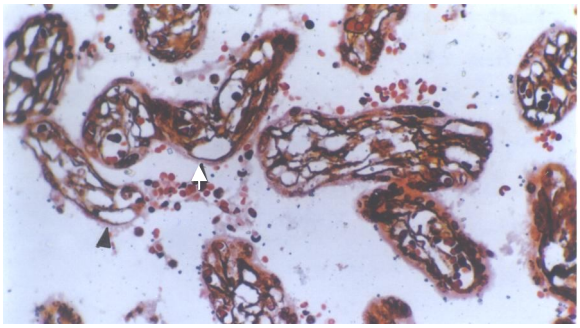

Figure-1: A 4 µm thick paraffin section stained with Gomori's Methanamine Silver of full term human placenta from Group A showing normal basement membarance of trophoblasts ×416

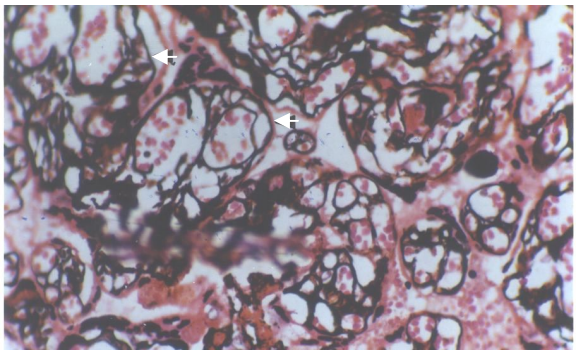

Figure-2: A 4 µm thick paraffin section stained with Gomori's Methanamine Silver of full term human placenta from Group "B" Showing normal basement membarance of trophoblasts against arrow head ×416

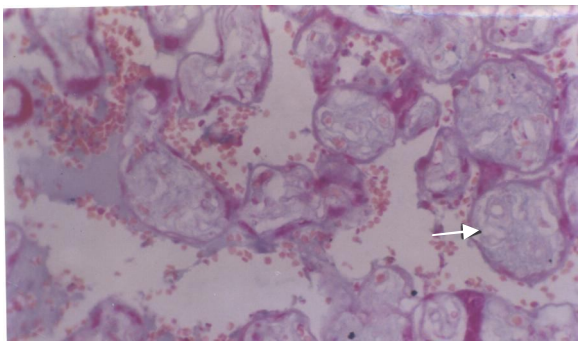

Figure-3: A 4 µm thick Mallory's trichrome stained paraffin section from Group-A Showing scanty chorionic villous collagen ×416

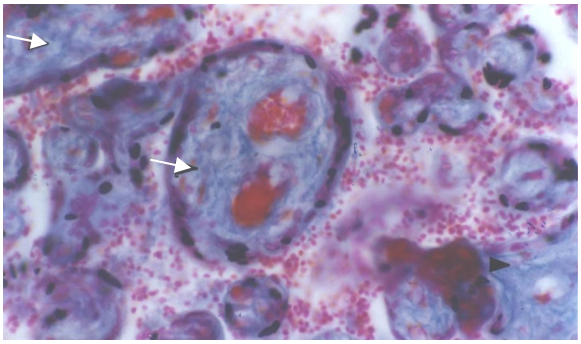

Figure-4: A 4 µm thick Mallory's trichrome stained paraffin section from Group-B (Snuff users) showing scanty chorionic villi collagen ×416

DISCUSSION

Placenta is an essential organ for exchange of materials between mother and fetus[8] Attachment of umbilical cord is mostly at the centre of foetal side of normal placenta.[9] Thickness of placenta depends on the length of stemp vill. Growth of placenta occurs in two phases, hyperplasia followed by hypertrophy.

As all the subjects were healthy females and there was no evidence of malnutrition and anaemia. This may be the reason of normal grass morphology of placentae in group A. These result correlates with the previous work done by Laga and associates[10] who demonstrated that the placenta of malnourished mothers had anatomic and morphologic alterations. Regarding the size (weight, diameter and central thickness) no significant decrease was found in placenta of snuff users when compared with normal placenta.

Normally aging (maturation) of placenta occurs during nine months period.[11] In placenta of snuff user's morphological changes consists, 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 high incidence of apoptosis in parenchymal cells of placenta. These findings are similar to those reported by Ellis *et al*[12] who reported atrophy and involution of human tissue and organs undergoing extensive apopotosis. They also

reported increased apoptosis in tissues undergoing ischemia. The results are also in accordance with those reported by Tominaga[13] who found extensive syncytial buds formation in hypoxic conditions of placenta.[14]

Snuff contains ground tobacco leaves, stalks and small amount of menthol.[15] Nicotine from tobacco is absorbed through oral mucosa and from the mucous membrane of GIT as small amount of snuff is also ingested during the process.[16] Nicotine triggers rapid elevation of plasma concentration of catecholamine resulting increased maternal blood pressure.[17] Nicotine crosses placental barrier and can effect the foetal circulation as well. Nicotine also reduces foetal and maternal vascular prostaglandin I2 (PGI2)[19] particularly in placenta and umbilical cord through cyclo-oxygenase inhibition.[20] Increased maternal blood pressure and inhibition of PGI2 by nicotine may lead to reduced uteroplacental perfusion[21]. In this way chorionic villi suffer hypoxia which affects the parenchyma of an organ.[18] Catecholamines also cause direct vasoconstrictive effect on placental vessels which further aggravates uteroplacental hypoxia.[19] In response to this hypoxia above-mentioned changes have been noted in parenchyma of snuff users' placentae.

Hypoxia and nicotine are stimuli for increased apoptosis in snuff user's placentae.[25] Due to increased incidence of apoptosis, large number of parenchymal cells (trophpplasts, endothelial cells) have been observed to be eliminated and replaced by fibrous tissue.[21] This fibrous tissue was synthesized by fibroblasts of villous stroma. Fibroblasts also take part in the synthesis of subtrophoblastic basement membrane.[23] In this way villous collagen increased in snuff user's placenta and increased collagen in villi effects the subtropoblastic basement membrane leading to increased thickness.[22]

Snuff user's placentae have shown highly significant increase in number of syncytial buds when compared with normal placenta. This morphological response to hypoxia leads to excessive loss of trophoblasts which produce hormones necessary for continuation of normal pregnancy. So the premature labour may result in snuff users.[24]

Increased villous collagen and increased thickness of subtrophoblastic basement membrane lead to increased thickening of placental barrier between foetal and maternal blood and this may in turn reduce the exchange of materials across placenta. This may result in low birth weight babies in snuff users.

On the basis of results of present study, it is concluded that use of snuff during pregnancy is as dangerous as smoking and may lead to pathological changes in micromorphology of human placenta resulting in abnormal outcome of pregnancy i.e. premature labour, abortions & low birth weight babies.

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