Original Article

Effects of Use of Smokeless Tobacco on Microstructure of Human Placenta

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Abstract

Objective: To understand the use and to see the effects of smokeless tobacco on placental microstructure.

Study Design: From June 2017 to December 2017 at Isra University Hyderabad

Methodology: Sixty full-term human placentae from normal and smokeless tobacco users were selected for this study. The placentae were grouped into two categories (Group A) smokeless and (Group B) control of 30 each. For the microscopic observations in the trophoblast of the placenta, the 4μm thick paraffin embedded segments were prepared and stained with H&E & Mallory's trichrome. The count of syncytial knots per unit area square and chorionic villus collagen per square unit area were observed with the help of ocular grid micrometry. The terminal villi were observed by H& E and oil immersion technique accordingly.

Results: The microscopic picture showed the marked changes in the placental morphology of smokeless tobacco user animals. The mean count of syncytial knots per square unit area (0.0625mm2), was 9.3 SD ± 1.3 in tobacco users and in the control group it was observed as 3.99 SD ± 1.4. The difference between the two groups was significantly higher (P< 0.001). The terminal villi appeared to be closely packed showing lesser intervillous space. The number of chorionic villi along with unnecessary collagen per unit area 0.0625mm2 in the section of placentae from group A was found to be 5.94 ± 0.32 whereas in group B it was much less i.e. 2.31 ± 0.16. This showed a significant difference between the two groups.

Conclusion: This study concluded that smokeless tobacco consumption during pregnancy produce significant changes in the placental morphology and may affect the outcome of pregnancy. It would cause the loss of trophoblasts and impairment of the placental blockade in the form of the number of syncytial knots, chorionic and terminal villi which can lead to hormonal imbalance, premature labor and low birth weight of babies.

Keywords: Placenta, Smokeless tobacco, Syncytial knots.

Cite this article as: Mughal F, Memon ZA, Qureshi SS. Effects of Use of Smokeless Tobacco on Microstructure of Human Placenta. J Soc Obstet Gynaecol Pak. 2019; Vol 9(1):3-8.

Introduction

The tobacco usage has raised in current years, particularly among adults and adolescent.[1] The use of tobacco is in two forms either smoke form or smokeless form can cause serious health problems.[2] Several adverse and serious human wellbeing consequences have been associated to use of smokeless tobacco including oral leukoplakia, gastrointestinal abnormalities, pancreatic, esophageal and oropharyngeal malignancy in

Authorship Contribution: [1]Drafting the work or revising it critically for important intellectual content, [2]Final approval of the version to be published, [3] participated in the acquisition and data analysis.

Funding Source: none

Conflict of Interest: none

Received: Jan 19, 2019

Accepted: Mar 24, 2019

addition to malignancies of the stomach.[3] Further potential adverse wellbeing consequences of smokeless tobacco can possibly comprise toxicity of the reproductive, cardiovascular, and immune systems.[4] In addition, its addiction can transfer substances across feto-placental membrane and affect pregnancy outcome.[2] Out of 3 hundred natural products within the smoke, the nicotine is the most hazardous and could influence the tissues of placenta. It also directly influences the foetus as it can easily lead to cross blockade of the placenta because of being highly fat soluble. Because of accentuation of premature and degenerative aging processes within placenta due to tobacco addiction, transfer substance through the fetomaternal membrane is adversely influenced which can possibly result in deleterious consequences on the outcome of pregnancy.[5]

Placenta, the primary site for gaseous and nutrient exchange from fetus to mother or vice versa, is a fetomaternal structure that comprises of two components (a) a maternal part. (b) a fetal part.[6]

The human placenta at term is a flattened discoidal mass with nearly oval or circular outline, however the shape is molded via a villi patch of chorionic sac left at last. [7]

The fetal portion, by the commencement of the 4th month of pregnancy, is turned into a chorionic frondosum and the maternal portion, is turned into decidua basalis.[8]

The placenta of human is at first convoluted when the initial villus stems are shaped, and develops into villus with the development of terminal villi generations. Maternal blood showers the chorion's surfaces that bounds the intervillous space and therefore the placenta is described as haemochorial.[7]

The materials exchange from mother to fetus and vice versa occurs at fetomaternal blockade that isolates maternal blood from fetal circulation into the intervillous space. These fetoplacental blockades comprise of endothelial cells in foetal vessels and their basement membrane, subepithelial basement membrane, villi's connective tissue and its layers of syncytiotrophoblasts and cytotrophoblast.[9]

This blockade allows hormones, nutritive substances, oxygen, water, and a few of the excretion products to circulate between mother and fetus. Functionally, the fetomaternal barrier is a most significant placental part.[10]

Syncytial knots or syncytiotrophoblastic knots are the syncytial nuclei's aggregates at the terminal villi's surface. In term placenta, most syncytial knots are believed to be entities from tangential segmenting whereas the minority are apoptotic knots, bridges, or syncytial sprouts. Syncytial knots are constantly there, growing with elevating gestational age, and can be helpful to assess villus maturity. Elevated syncytial knots are correlated with uteroplacental malperfusion conditions and are significant in placental checkup. Term placentas (37-40 weeks) exhibited averagely 28% of syncytial knots.[11]

As a fact raised syncytial knotting occurs due to villi's response towards hypoxia, where villi try to grow their superficial area to ease the exchange of oxygen with the blood of the mother. A rise noticed in syncytial knots is frequently correlated with oxidative damage and uteroplacental hypoperfusion.[12]

Syncytial knots were noticed further commonly in smokers' placentas, confirmed via the literature[13] and proposes that oxidative damage and malperfusion are raised among smoking mothers contrasted to controls.[14] This study extended these outcomes herein to exhibit their existence in placental sections from gravidae who were addicted to smokeless tobacco during pregnancy.

Methodology

In the present study the total of 60 full-term human placentae from smokeless tobacco user, and control were used. The placentae were grouped into two categories (Group A) smokeless and (Group B) control of 30 each.

The specimens were taken from the Obstetrics and Gynaecology, Department, Unit II, Liaquat University of Medical & Health Sciences, and from the department of Gyneacology and Obstetrics and Isra University Hyderabad. The placentae were fixed in 10% formaldehyde. Further processing and practical work was carried out in the Department of Anatomy and, Postgraduate Laboratory of Basic Medical Sciences Isra University.

The Placentae were rinsed well under running tap-water to eliminate the blood clots from the Placental surface. Placental Umbilical cords were cut 4cm above from attachments at foetal surface and foetal vessels were squeezed gently to drain the blood through umbilical vessels. The placental membranes were detached and various microstructural parameters were recorded.

Before the specimen's collection from labour room, the the examination of patients was performed in the ward as well as brief histories were taken about their tobacco habit.

All the patients enrolled in this study were healthy looking primary gravid/multiparous mothers with age ranging from 18 to 35 years.

Those patients who were found with obstetric abnormalities, i.e. congestive heart failure, abruption placenta, jaundice and twin pregnancy were excluded from the study. All the obtained Placentae were of elective C section or normal vaginal deliveries.

The placentae were grouped into two categories.

Group A. comprising 30 Placentae from females addicted to smokeless tobacco.

Group B. comprising 30 Placentae from females that were not complicated via any disorder or addiction of mothers to any substance.

For microscopy, in each group the placentae were studied for:

I. Number of chorionic villus collagen
II. Average quantity of syncytial knots per unit area
III. Terminal villi

Tissue Processing for Sectioning

Paraffin Sections: Placentae set within formalin 10% were processed for embedding routine paraffin. Tissue pieces 2x2 cm measuring from standard site, i.e. 2 cm from periphery were taken. 4 micron thick units were cut on rotary microtome from the middle of each specimen, and were set on sterile gelatinized slides, stained with Mallory's trichrome, H&E.

Micrometry Calibration of Reticle or Ocular Grid: Reticle was used to count the chorionic villus collagen and syncytial; knots per square unit area. The reticle was fixed into the microscope eyepiece. The field size outlined via the reticle was calibrated by stage micrometer. The ocular grid's two opposing sides (i.e. breadth and length) were consecutively coincided with stage micrometer having a scale of 1mm length distributed into 100 parts. Under 40x objective, each small box of the reticle coincided with $0.025mm^2$ length of stage micrometer. To calculate the area the two sides of a small square was $0.025x0.025=0.000625mm^2$ and for 100 small square of the reticule, it was $0.000625x100=0.0625mm^2$ which was the total unit area of 100 squares occupied by the reticle.

A reticle fitted in the X 10 eyepiece of a Olympus CH40 microscope, the objective lens of X 40 was used to get a total magnification of X 400. Syncytial knots and villi per field were counted in 4 randomly chosen fields and populations were expressed as mean number of syncytial knots or villi per unit area (i.e. $0.0625mm^2$) of placenta. In order to bring syncytial knots and chorionic villi to focus on the depth of the field was changed from time to time. The mean number of syncytial knots and chorionic villi were calculated from the total count of the respective region.

Haematoxyline and Eosin stain for Number of Syncytial Knots / unit area: To count the number of syncytial knots per unit area in both groups A and B, the heamtoxyline and Eosin (H&E) stained sections were utilized. The mean number of syncytial knots were measured by the ocular grid reticle technique.

Mallory's Trichome stain for the amount of chorionic villi: The amount of collagen in the stroma of chorionic villi was observed by the Mallory's Trichme stain and villus collagen was measured by the help of reticle.

Haematoxyline and Eosin stain for observation of terminal villi: The H& E stain was used to observe the terminal villi and intervillous spaces in the sections of placenta.

The statistical significance of the variance between means of different parameters and between various groups was assessed by student "t" test and data analysis was done by SPSS Version 24.

Results

Microscopic features of placentae in both group A and B were observed with various stains and magnification levels. These features included syncytial knots, chorionic villi with collagen and terminal villi.

Terminal Villi: Terminal villi appeared to be closely packed showing lesser intervillous space in the H&E section taken from group A placentae as compared to seen in sections from group B placentae (Figure 1 & 2).

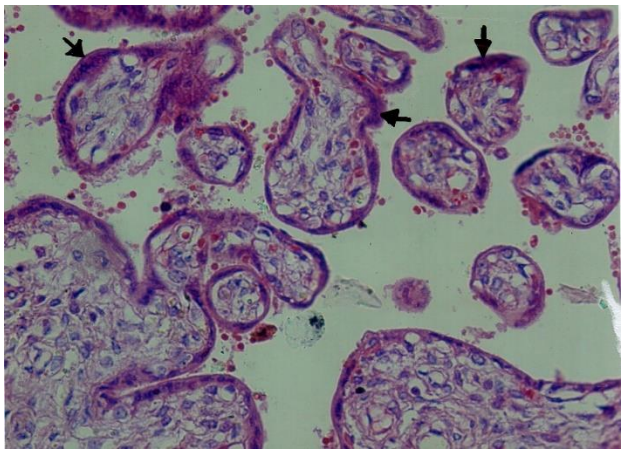

Figure 1. A photomicrograph of a 4μm thick H&E stained section of full term normal human placenta from control (group B) showing terminal villi with scanty syncytial knots X 400 (shown by black arrow heads)

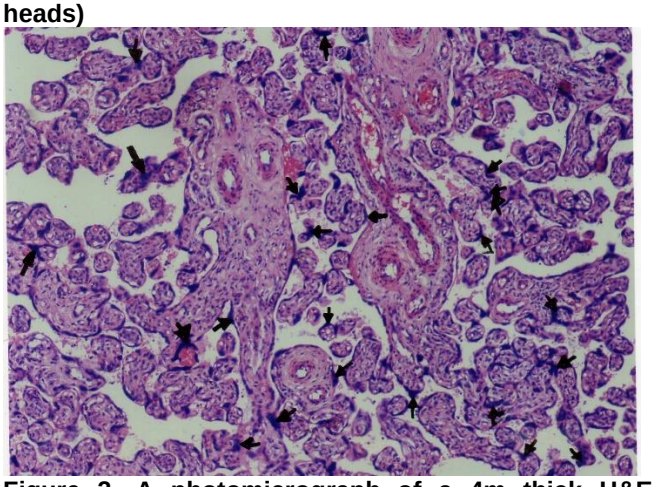

Figure 2. A photomicrograph of a 4m thick H&E stained section of smokeless tobacco user human placenta from group A showing prominent and numerous syncytial knots. Villi are closely packed with less intervillous distance at magnification power X 100 (shown by black arrow heads)

Syncytial Knots / 0.0625mm^2: The syncytiotrophoblasts heap up at certain places and form the syncytial knots, which were observed and counted in both groups. The mean number of knots observed in group A were 9.3±1.3/0.0625mm^2 whereas in group B was only 3.99±1.4/0.0625mm^2. (table I) This shows a significant difference ($p<0.05$) in two groups (Table II) (Figure 3).

Table I: Comparison of number of syncytial knots per unit area (0.0625mm^2) between group A & B

Groups	Mean n=30	Std. Deviation	p Value
Group (A)	9.300	± 1.30	< 0.001
Group (B)	3.991	± 1.41	< 0.001

Table II: Comparison of chorionic villi with excessive collagen per unit area (0.0625mm^2) between group A & B

Groups	Mean n=30	Std. Deviation	p Value
Group (A)	5.94	± 0.32	< 0.01
Group (B)	2.31	± 0.16	< 0.01

Chorionic Villi with Excessive Collagen / 0.0625mm^2: In the sections stained with mallory's trichome, the amount of collagen in the stroma of chorionic villi was observed. The number of chorionic villi with excessive collagen per unit area 0.0625mm^2 in the section of placentae from group A was found to be 5.94 ± 0.32 (Figure 5) whereas in group B it was much less i.e. 2.31 ± 0.16 (Figure 4). This showed a significant difference in two groups (Table II.)

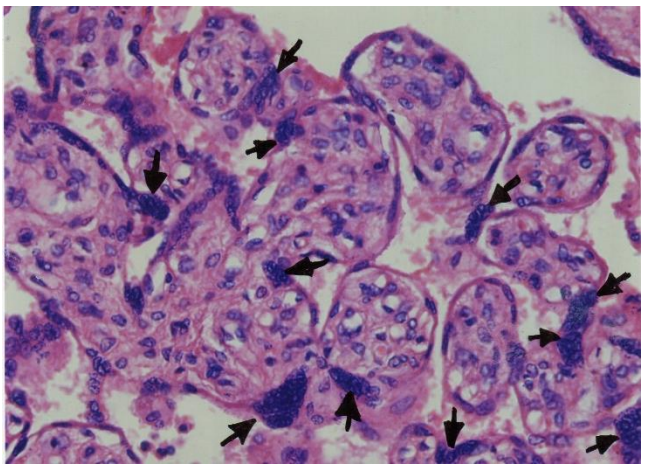

Figure 3. A photomicrograph of 4μm thick H&E stained section of full term smokeless tobacco user's human placenta (group A), showing reduced number of villus spaces, and numerous number of syncytial knots against the arrow heads X 400 (shown by black arrow heads)

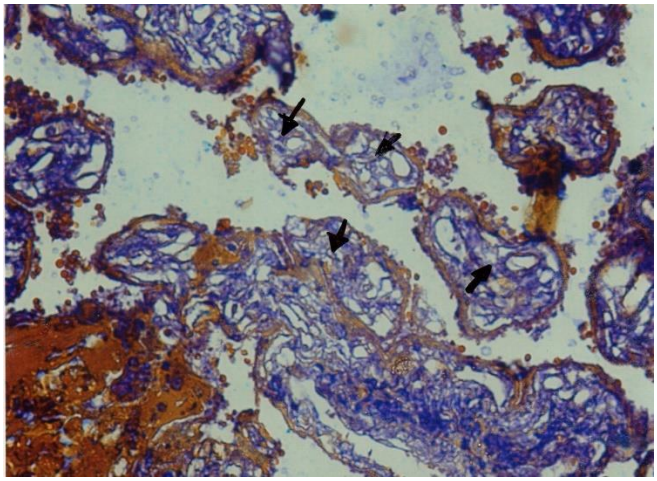

Figure 4. A photomicrograph of a 4µm thick Mallory trichrome stained section of full term normal human placenta control (group B), normal amount of collogen with in terminal villi X 400 (shown by black arrow heads)

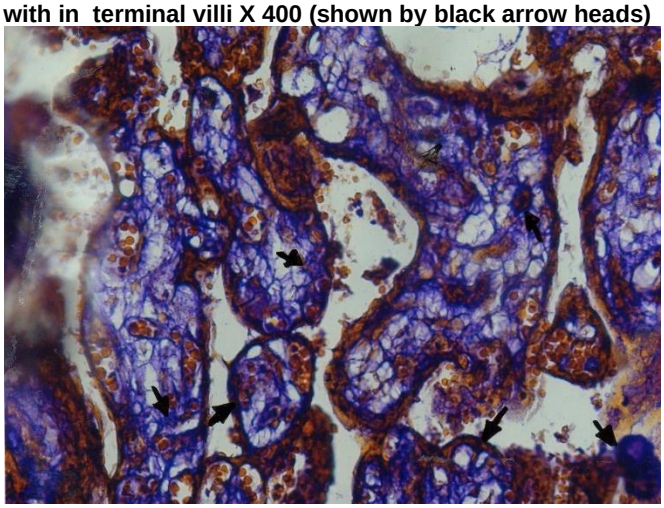

Figure 5. A photomicrograph of a 4µm thick Mallory trichrome stained section of full term smokeless tobacco user's human placenta (group A), showing excessive collogen and large number of terminal villi x 400 (shown by black arrow heads)

Discussion

Placenta is a vital organ for metabolites and nutrients exchange between foetus and mother. The endothelial nature of the surfacing syncytiotrophoblast as well allows directional hormonal secretions, cytokines and growth factors into the blood of the mother to effect fetal growth and substrate supply. While placental function is vital at every stage of pregnancy, transportation of nutrient generally demands upon chorioallantoic villi, and in particular, upon villus trophoblasts, are greatest as the fetus mainly grows in body weight in the course of the 3rd trimester of gestation.[15]

Developmental variations in normal Placentae in the course of 9 months of its intrauterine presence are believed to be an organ's aging process with short life span.[15] These changes are intensified in response to placental hypoxia and include raised stromal fibrosis, more and enlarged syncytial knots per unit area, unnecessary thickening of the membrane of subtrophoblastic basement, and very high occurrence of apoptosis within placental parenchymal cells.[5,16]

Loss of trophoblasts leads to the development of syncytial knots and increased number and size of syncytial knots is a significant morphological feature of tobacco users placentae when compared with normal placentae. In this study, the mean number of syncytial knots seen in Group A placentae is 9.30 ± 1.3 per 0.0625mm^2 as compared to be seen in group B i.e. 3.99 ± 1.4 per 0.0625mm.^{2} This increase in number is little less that than seen in placentae of smokers as observed by Ashfaque et al 2003.[5]. This is because of additional hypoxic effects of carbon monoxide in smoking along with nicotine.

These morphological changes in response to hypoxia represent a useful accommodation in order to lower the distance which must be traveled by oxygen between foetal and maternal plasma however raised loss of trophoblasts in terms of these syncytial knots can possibly result in premature labor and hormonal imbalance.[17] Raised villus collagen can possibly result in raised thickening of placental blockade between foetal and maternal blood and this can possibly, in turn, lower the materials exchange across the placenta.[18]

The villus trophoblasts' apoptosis elevates with pregnancy progression[19] and insults that yield villus injury can possibly not be accompanied via a balancing rise in cytotrophoblast differentiation and proliferation.[20]

Imbalances of repair and injury and villus tree's mal-development are placental characteristic in IUGR. This inclines towards syncytiotrophoblast's depletion with a subsequent limitation on secretory functions and controlled transport.[21]

Conclusion

It was concluded that consumption of smokeless tobacco during the pregnancy produce significant

changes in the placental morphology and may affect the outcome of pregnancy. It would cause the loss of trophoblasts and impairment of the placental blockade in the form of the number of syncytial knots, chorionic and terminal villi which can lead to hormonal imbalance, premature labor and low birth weight of babies.

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