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Comparison of Vasculosyncytial Membrane Thickness at Different Gestational Periods

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Abstract Vasculosyncytial membrane is the only structural barrier between maternal and fetal circulation found in the wall of the terminal chorionic villi. Its normal function is essential for the survivability of a growing fetus. Understand the role of the placenta during fetal growth; it is necessary to know changes of vasculosyncytial membrane with gestational age. This study carried out on total ninety products of conception and placenta, and all the collected samples divided into three groups. One group included 30 products of conception from the first-trimester pregnant woman, and another two groups included a total of 60 placentae from the second and third-trimester pregnant woman. All the placentae were fixed in 10% formol saline for 48 hours, after fixation two tissue blocks taken from each specimen. After tissue processing and staining, the histomorphological changes studied among three groups under the light microscope — statistical analysis done by ANOVA test. In the first-trimester placenta, vasculosyncytial membrane was absent. In the second trimester, it ranged from 5.1 – 15.00 µm and the mean ± SD was 8.54 ± 0.84 µm. In the third trimester, it ranged from 1.90 – 4.70 µm, and the mean ± SD was 3.12 ± 0.73 µm. Vasculosyncytial membrane thickness significantly reduced with the aging of the placenta. The study findings suggest placental tissue continued to grow until term. Rate of exchange of gas and other nutrients supposed to be more with the advancement of pregnancy.

Keywords: *vasculosyncytial membrane, chorionic villi, diffusion distance, placenta*

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1. Introduction

Chorionic villous is the structural and functional unit of the placenta. These are the extra-embryonic mesodermal projections of fetal tissue, consists of two cellular layers: the outer syncytiotrophoblast, and this is in direct contact with maternal blood within the intervillous space, and the inner cytotrophoblast. A series of histological changes occur during the development of chorionic villi first step being the formation of primary villi by local proliferation of cytotrophoblast that penetrates syncytiotrophoblast at the end of the second week of gestation. The next step is the formation of secondary villi, where mesodermal cells penetrate the core of primary villi. The final step is the formation of tertiary villi when blood vessels appear within the mesodermal core at the end of the third week of gestation [1]. Although individual villi undergo considerable branching, most of them retain the same basic structural plan throughout pregnancy [2].

As the chorionic villi mature, there is a marked reduction of cytotrophoblast. So, at term, only a single layer of syncytiotrophoblast separates the maternal blood and fetal capillary [3] and functionally the syncytiotrophoblast and fetal capillary act as a single unit, called vasculosyncytial membrane that is the only physical barrier between fetal and maternal blood [4]. The most significant properties of the vasculosyncytial membrane are the maintenance of exchange surface area and sufficient diffusion distance of fetomaternal surfaces [5].

2. Materials and Methods

Study design: Cross-Sectional, analytical type of study.
Place of study: Department of Anatomy, Dhaka Medical College, Dhaka
Study sample: Product of conception of first trimester and placenta of the second and third trimester.
Sample size: this study was carried out on a total of 90 placentae (each 30 from each trimester of pregnancy). It calculated by the following equation,

$$n = \frac{\left(z_\alpha + z_\beta\right)^2 \times \left(\sigma_1{}^2 + \sigma_2{}^2\right)}{\left(\mu_1 - \mu_2\right)^2}$$

Here,
n = Sample size

$z_\alpha = z$ - Value of standard normal distribution at a given level significance
$z_\beta = z$ - Value of standard normal distribution at a given power.
σ_1 = SD of one group
σ_2 = SD of another group
μ_1 = Mean of one group
μ_2 = Mean of another group

Sampling technique: Convenient and purposive sampling.

Selection of sample: The study was carried out on a total of 90 human products of conception and placentae from adult Bangladeshi women. Thirty products of conception were taken from pregnant women whose gestational period was 6th to 12th weeks and came for Menstrual Regulation (MR) due to failure of the contraceptive method. Sixty placentae were taken from pregnant women whose gestational period was 13th to 40th weeks. Gestational age was calculated by dates of last menstrual period or by ultrasound measurement of the biparietal diameter. The selection of the study group was made based on diagnosis by registered doctors and the hospital records.

Collection of samples: All products of conception were collected from the pregnant woman whose menstrual regulation (MR) was conducted through manual vacuum aspiration from MR clinic. All placentae were collected from the pregnant woman whose deliveries were conducted through normal vaginal delivery or caesarian section from the labour room and operation theatre of Obstetrics and Gynecology Department of Dhaka Medical College Hospital. Each subject was informed about the entire plan, implication of the study. Written consent was taken from them without exploiting any of their weaknesses or without creating any undue pressure. They enjoyed the freedom to withdraw themselves from any part of the study. All placentae and products of conception were collected in plastic jars within hours after delivery. Each plastic jar was marked by a label containing the name, age, and period of gestation and date of collection. Samples were taken to the Department of anatomy, Dhaka medical college.

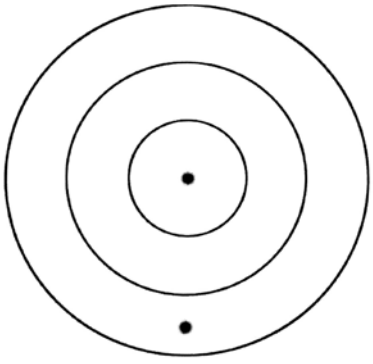

Figure 1. Diagrammatic representation of the division of the placenta into three zones showing two positions from which tissue was taken for microscopic study

From all products of conception, chorionic tissue was identified by its gross characteristics. Chorionic tissues were slender, delicate, white, and leaf of a fern like papillary fronds [6]. Then the blood clots were removed very carefully. Tissue blocks were made from each of the chorionic samples. Each placenta was placed on a flat tray and membranes were trimmed from the margins of the placenta, the umbilical cords were cut, keeping 2 cm of it with the placenta. The blood clots were removed carefully without damaging the placental tissue and gently cleaned with cotton. The placenta then cleaned with tap water until clear water comes out. From each placenta, two tissue blocks of full-thickness were taken from the central portion of cotyledon for microscopic examination, one from central and another from the peripheral position of the placenta (Figure 1). All tissue blocks were taken from the area, which appeared normal and least pathologic [7].

Grouping of the sample: All samples were divided into three groups as Group A, Group B, and Group C according to gestational age (Table 1)

Group A: This group comprises of products of conception that was collected from 6 to 12 weeks of aborted material.

Group B: This group comprises of placental tissue that was collected from 13 to 27 weeks of the delivered placenta.

Group C: This group comprises of placental tissue that was collected from 28 to 40 weeks of the delivered placenta.

Table 1. Showing grouping of sample and their size

Group	Sample size
Group A	30
Group B	30
Group C	30

Equipment for measurement:

1. Binocular Microscope (CX 21, Olympus, Tokyo, Japan)
2. Olympus optical stage micrometer
3. Olympus optical crossed scale ocular micrometer
4. Zeiss Integrating Eyepiece Disc 1
5. Sony DSC W100 digital camera (Tokyo, Japan)

Preparation of specimen: The tissue blocks were fixed into 10% formol saline for 48 hours. Then the tissues were washed in running tap water; dehydration was done with ascending grades of alcohol, cleared with xylene, infiltrated and embedded in paraffin. Paraffin blocks were cut at 6μm thickness and were stained with hematoxylin and eosin (H&E) stain.

Methods of measurements: Vasculosyncytial membrane thickness was measured by crossed scaled ocular micrometer.

The thickness of the vasculosyncytial membrane was measured in cross-sections of the terminal villi. Terminal villi were recognized as smallest villi lined with trophoblast contain highly coiled fetal capillaries and few stroma, and intervillous space contains maternal blood around the villi. Three terminal villi were selected from each field by stratified random sampling. Then the thickness of vasculosyncytial membrane was measured in micrometer by using crossed scaled ocular micrometer at high magnification ×100 objective ×10 eyepiece of the light microscope (Figure 2). Before that, ocular micrometer was calibrated with the stage micrometer. So, vasculosyncytial membrane of a total of 30 villi was measured. As two slides were taken from each placenta, a total of 60 villi were measured, and then the mean was calculated.

Ethical clearances: The research work was approved by the Ethical Review Committee (ERC) of Dhaka Medical College, Dhaka

Data processing and analysis: All data were checked and edited after collection. Later the data were statistically analyzed by a software package, SPSS for Windows (Version 24.0), keeping the objective of the study in view. Statistical test ANOVA was done.

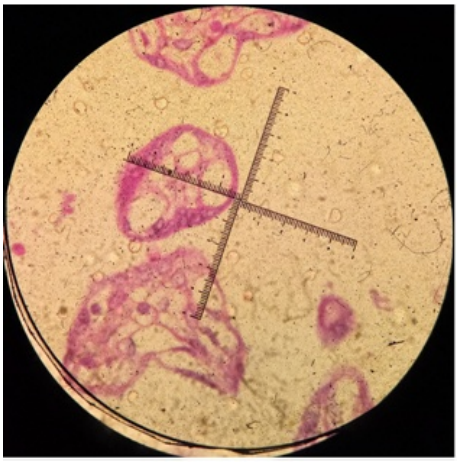

Figure 2. Photograph of a placenta while looking throw an integrating eyepiece marked with a crossed scale, which is used to measure the thickness of vasculosyncytial membrane, trophoblast, and diffusion distance from fetal to the maternal circulation. At high magnification (×100 objective ×10 eyepiece) by routine hematoxylin and eosin (H&E) stain

Data were expressed as Mean ± Standard deviation (± SD) as descriptive statistics among the three groups. Statistical significance was accepted at the p-value equal to or less than 0.05 ($p \leq 0.05$).

3. Result

Vasculosyncytial membrane thickness of first, second, and third-trimester placenta:

In the first-trimester placenta, the vasculosyncytial membrane was absent. In the second trimester placenta, vasculosyncytial membrane ranged from 5.1 – 15.00 µm and the mean ± SD was 8.54 ± 0.84 µm. In the third trimester placenta, vasculosyncytial membrane ranged from 1.90 – 4.70 µm and the mean ± SD was 3.12 ± 0.73 µm. Statistically, a significant difference was observed among 1st, second, and third-trimester placenta in the mean trophoblastic thickness ($p < 0.00$) (Table 2 & Figure 3)

Table 2. Vasculosyncytial membrane thickness in 1st,2nd,3rd trimester placentae

Trimester	Vasculosyncytial membrane thickness (µm)
	Mean ± SD
1st (n = 30)	0.00 ± 0.00 (0.00 – 0.00)
2nd (n = 30)	8.54 ± 0.84 (5.1 – 15.0)
3rd (n = 30)	3.12 ± .73 (1.90 – 4.70)
1st vs. 2nd 2nd vs. 3rd 1st vs. 3rd	p- Value 0.00* 0.00* 0.00*

4. Discussion

Vasculosyncytial membrane is an essential element of the placenta. The functional integrity of placenta depends on the normal structure of Vasculosyncytial membrane. It is necessary to know the typical histological structure of Vasculosyncytial membrane for identification of any pathology related to the placenta.

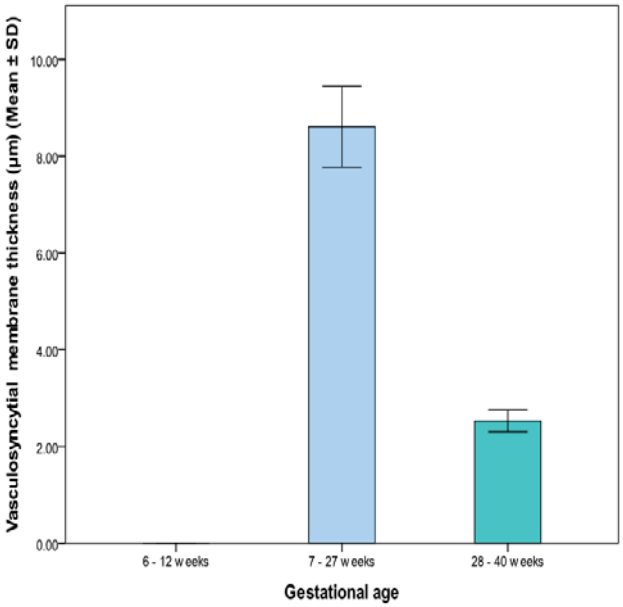

Figure 3. Vasculosyncytial membrane thickness in 1st,2nd,3rd trimester placentae

The present study was carried out on a total of 30 human products of conception and 60 placentae from adult Bangladeshi pregnant women for histological examination. In this discussion, the variation of Vasculosyncytial membrane was discussed.

It is observed by reviewing of literature that many works have been carried out on Vasculosyncytial membrane thickness in various disease conditions like diabetes mellitus, hypertension, hyperthyroidism, hypothyroidism, eclampsia, pre-eclampsia, molar pregnancy, anemia, monosomy, trisomy and various infections like TORCH infection at different periods of gestation. The histological structure of chorionic villi could be changed in above-mentioned disease conditions. However, so far it is known that there is no published work conducted on Bangladeshi population on the histomorphological study of the placenta at different gestational periods.

The mean vasculosyncytial membrane thickness in the second and third-trimester placenta of the present study was 8.54 ± 0.84 µm and 3.12 ± 0.73 µm, respectively. A statistically significant difference ($p < 0.000$) was observed between 1st, second, and third-trimester placenta in mean trophoblastic thickness.

Mean vasculosyncytial membrane thickness in third term placenta of normal and hypertensive mother 2.78 ± 0.029 and 3.16 ± 0.075 [8]. A statistically significant difference ($p < 0.001$) was observed between those two groups. From the result of the present study, it was also assumed that mean vasculosyncytial membrane thickness increase in hypertension.

5. Conclusion

In the present study, it has been found that the vasculosyncytial membrane became thinner with increasing gestational age.

6. Recommendation

Further studies of chorionic villi and placenta using different special stains like Masson's trichrome, toluidine blue, periodic acid-Schiff (PAS) are recommended.

Ultrastructural and histochemical study of chorionic villi and placenta are also recommended.

A similar study on gestational hypertension, gestational diabetes, anemia, spontaneous abortion compared with the present study recommended.

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