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Variation of Diffusion Distance from Foetal to Maternal Circulation at Different Gestational Periods

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ABSTRACT

Diffusion distance varies according to the gestational age of a mother. Moreover, this diffusion distance must be within a physiological range. Thus, the survivability of a growing foetus depends on this distance. For this study, 90 products of conception and placenta were collected and divided into three groups based on the trimester of pregnancy, and each group included 30 samples. Two tissue blocks were collected from each specimen after the sample was fixed with 10% formol saline for 48 hours. Afterwards, with further processing of the tissue, followed by H&E staining, the diffusion distance was measured among the three groups by using crossed sealed ocular micrometre under the light microscope. The ANOVA test was considered for further statistical analysis. In the first trimester, the placenta diffusion distance from foetal to maternal circulation ranged from 53.20 – 68.30 (µm), and the mean ± SD was 60.46 ± 3.58 (µm). In the second-trimester placenta, the diffusion distance of placenta from foetal to maternal ranged from 23.20 – 45.30 (µm), and the mean ± SD was 36.05 ± 6.01 (µm). In the third-trimester placenta, diffusion distance from foetal to maternal ranged from 1.80 – 2.90 (µm) and the mean ± SD was 2.52 ± 0.22 (µm). Diffusion distance significantly reduced with the ageing of the placenta ($p < 0.00$). With the help of this result, we can assume that the exchange of nutrients and gases proportionally increases with the advancement of pregnancy.

INTRODUCTION

The foetal tissue consists of mesodermal projections known as chorionic villous, which are the structural unit of the placenta. Chorionic villous are extra-embryonic and comprises of the syncytiotrophoblast, and the cytotrophoblast. Syncytiotrophoblast and cytotrophoblast are the outer and inner cellular layers, respectively. Syncytiotrophoblast lies within the intervillous space, which is a conjunction for the syncytiotrophoblast to be in close proximity with the maternal blood. For the formation of mature chorionic villi, subsequent histological changes occur during the development, starting from primary villi formation, with there being cytotrophoblast propagation which perforates syncytiotrophoblast near the end of the second week of gestation, followed by the development of secondary villi. During the secondary villi development, the extra-embryonic mesenchyme penetrates the primary villi centre. Finally, the appearance of blood vessels near the mesenchymal centre leads to the formation of tertiary villi during the end of the third week of gestation[1] (Figure 1). However, each villus branch out with the advancement of pregnancy but basic structural architecture remain almost same throughout the pregnancy[2].

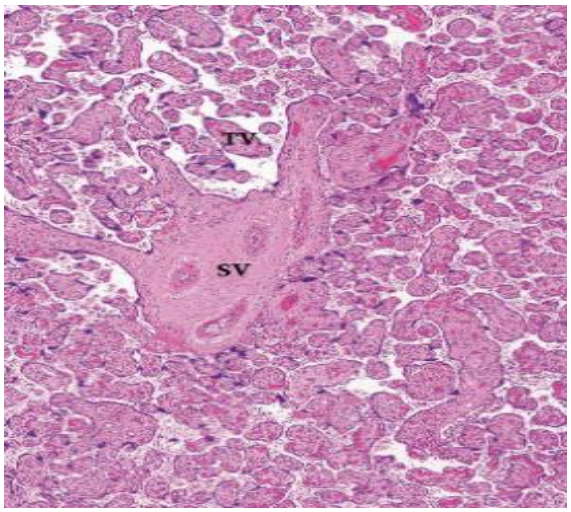

Figure 1 Micrograph of the full-term placenta at low magnification (SV indicates stem villi; TV indicates terminal villi)[3]

Mature chorionic villi consist of a little amount of cytotrophoblast. The maternal blood is isolated from the foetal capillary by a single syncytiotrophoblast layer after a certain period[4]. However, the syncytiotrophoblast and foetal capillary can be considered as an individual unit, in terms of their functionality, known as the vasculo-syncytial membrane that is the only structural barrier between foetal and maternal blood[5] (Figure 2). Three principal morphological characteristic features can describe the vasculo-syncytial membrane. Firstly, the capillary of the foetal blood vessel become widely dilated, up to 40 – 50 µm in diameter[6,7]. Secondly, basal lamina of the foetal capillary intimate with the syncytiotrophoblast with intervening littlest stromal tissue, and thirdly the syncytiotrophoblast is very thin and anuclear. Therefore, there may be an overall reduction in the width of the vasculo-syncytial membrane up to 1 – 2 µm. Consequently, vasculo-syncytial membranes are generally considered the significant sites of diffusional exchange between mother and foetus[8,9].

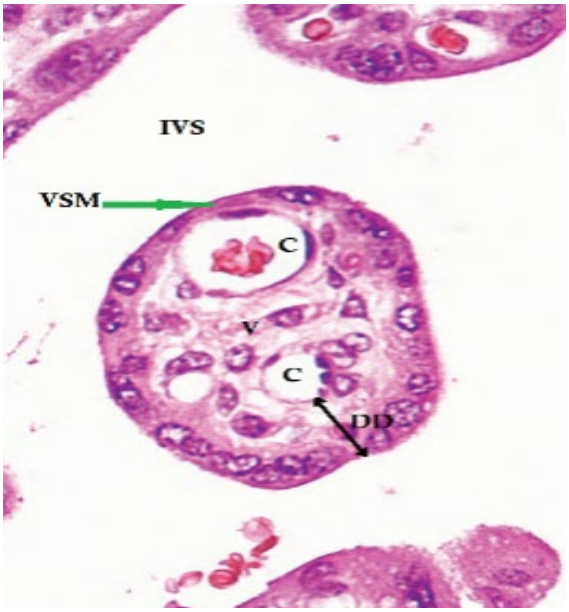

Figure 2 Micrograph of full-term placental terminal villi at high magnification (IVS indicates intervillous space, VSM indicates vasculo-syncytial membrane, C indicates foetal capillary, DD indicates diffusion distance)[10]

The vasculo-syncytial membranes are developed due to protrusion of the foetal capillaries into the trophoblastic layer[11]. Due to high transmural pressure and localised proliferation of endothelium, the foetal capillaries become dilated and make close contact with trophoblast[12]. The most important properties of the vasculo-syncytial membrane are the maintenance of exchange surface area and sufficient diffusion distance of foeto-maternal surfaces[13]. A recent study shows that there is a consistent relationship between the volume of the foetal capillaries and the mean thickness of the vasculo-syncytial membranes[14]. It is essential to know about the chronological changes of diffusion distance from foetal to the maternal circulation of the placenta. The slight alteration may cause intense foetal damage. For example, increasing thickness of vasculo-syncytial membrane results in increased diffusion distance may lead to maternal pre-eclampsia, which may cause maternal and foetal death. Diffusion distance from foetal to maternal circulation vary at different gestational periods. So, it can be a critical anatomical data for correlation with the pathology of the placenta.

MATERIALS AND METHODS

This was a cross-sectional, analytical type of study. This study was conducted in the Department of Anatomy, Dhaka Medical College, Dhaka from January 2016 to December 2016. Ethical permission was approved by the Ethical Review Committee (ERC) of Dhaka Medical College, Dhaka MEU-DMC/ECC/2016/189. Total of 90 samples was collected from adult Bangladeshi women. It calculated by the following equation,

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

where,

n (Sample size) = 30

z_α (z - Value of standard normal distribution at 99.99% confidence level) = 3.89

z_β (z - Value of standard normal distribution at 2.33 at power) = 0.99

σ_1 (SD of one group) = 1.4

σ_2 (SD of another group) = 1.5

μ_1 (Mean of one group) = 1.5

μ_2 (Mean of another group) = 1.66

In this study, the mean percentage volume of fibrin was used for the calculation of sample size measurement[15]. The sampling technique was stratified random sampling. The participants provided informed consent without being exploited or forced. Either date of the last menstrual period or ultrasonic measurement of the biparietal diameter was used to calculate gestational age. Depending on the conclusion by enrolled specialists and the medical clinic records, the selection of the study group was made and divided into three examination groups named as Group A (6 to 12 weeks of aborted material), Group B (13 to 27 weeks of the conveyed placenta), and Group C (28 to 40 weeks of the conveyed placenta).

From all products of conception, chorionic tissue was recognised by its gross attributes. Chorionic tissues were thin, fragile, white, and a leaf of a fern-like papillary fronds[16]. The tissue block was prepared out of each of the chorionic samples, after careful removal of blood clots from it. The placental membranes were trimmed off the margins, and 2 cm of the umbilical string was kept with the placenta after cutting rest of it. At that point, faucet water was used to clean the placenta and later on, it was completely dehydrated. For microscopic examination, two full-thick tissue blocks were taken from each specimen; one from the central portion of cotyledon and then another from the peripheral position of the placenta. The region of the placenta seemingly having minimal pathology and standard features were selected for harvesting tissue blocks[17].

Then the tissue blocks were successively subjected to fixation with 10% formol saline for 48 hours, washing in running tap

water, dehydration with ascending grades of alcohol, clearing with xylene, infiltration and embedding in paraffin. After cutting the paraffin blocks at 6µm thickness, paraffin sections are then stuck to glass slides, deparaffinised, and stained with Haematoxylin and Eosin (H&E).

Diffusion distance from foetal to maternal circulation was measured in terminal villi cross-sections by Olympus optical crossed scaled ocular micrometre. Stratified random sampling was used to select three terminal villi from each field. With the aid of Olympus optical crossed scaled ocular micrometre, the diffusion distance from foetal to maternal circulation was measured (in micrometre) at 100×10 times magnification at the light microscope (Figure 4) after calibration with the Olympus Optical Stage micrometre.

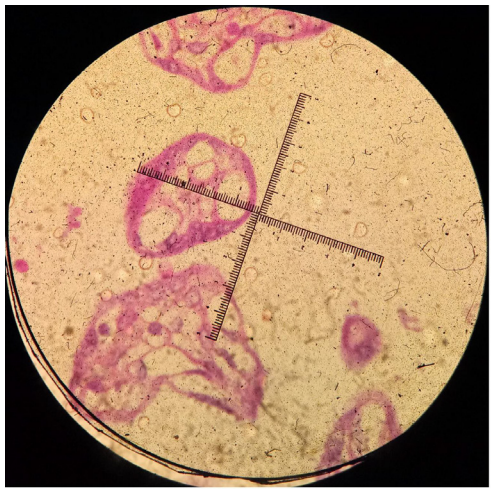

Figure 4 Photomicrograph of H&E stained placenta at 100×10 times magnification (view through an integrating eyepiece marked with a crossed scale used to measure the diffusion distance from foetal to the maternal circulation)[18]

Data Analysis

The data were statistically analysed after collection and checking of all data by a software package, SPSS for Windows (Version 24.0), keeping the objective of the study in view. Statistical test ANOVA was done to achieve the variation of diffusion distance at different trimester placenta. Data were expressed as Mean ± Standard deviation (± SD) as descriptive statistics among the three groups. Statistical significance was acknowledged at the *p*-value equal to or less than 0.05 ($p \leq 0.05$).

RESULTS

In group A (1st-trimester placenta), diffusion distance from foetal to maternal circulation ranged from 53.20 – 68.30 µm. There was a vast distance from foetal endothelium to intervillous space, and foetal capillaries are comparatively narrow and less in amount; chorionic villi contain lots of mesodermal tissue. Stem villi are comparatively more than the terminal villi. Cytotrophoblast and syncytiotrophoblast were visible. In group B (2nd-trimester placentae), diffusion distance from foetal to maternal ranged from 23.20 – 45.30 µm. In group C (3rd-trimester placentae), diffusion distance from foetal to maternal ranged from 1.80 – 2.90 µm (Table 1). Numerous terminal villi with few mesodermal tissues were found. Capillaries were widely dilated; the basement membrane of capillary endothelium fused with syncytiotrophoblast and trophoblastic tissue was not visible. Aggregation of syncytial nuclei (syncytial knot) was found at the syncytiotrophoblast. Mean diffusion distances were statistically significant ($p < 0.00$) among the three groups of placentae.

Table 1 Mean diffusion distance in different groups (n = 90)

Group	Vasculo-syncytial membrane thickness (μm)
	Mean ± SD
Group A (n = 30)	60.46 ± 3.58 (53.20 – 68.30)
Group B (n = 30)	36.05 ± 6.01 (23.20 – 45.30)
Group C (n = 30)	2.52 ± 0.22 (1.80 – 2.90)
Statistical analysis	***p*-value**
Group A vs Group B	0.00*
Group A vs Group C	0.00*
Group B vs Group C	0.00*

*Significant as $p < 0.05$

DISCUSSION

The study aimed to provide data on the growth of the placenta by analysing the different histomorphological structure. The mean diffusion distance in first, second and third-trimester placentae of the present study were 60.46 ± 3.58 μm, 36.05 ± 6.01 μm and 2.52 ± 0.22 μm respectively. A statistically significant difference ($p < 0.00$) was observed between first, second and third trimester placentae in mean diffusion distance.

Kaufmann and Scheffen[19] found mean diffusion distance in first, second and third-trimester placenta 55.9 ± 4.74 μm, 27.7 ± 5.84 μm, and 1.12 ± 0.73 μm respectively. A statistically significant difference ($p < 0.05$) was observed between the first, second and third-trimester placenta in mean diffusion distance. Though the values of the present study were different from the findings of the study mention above, mean diffusion distance was increased as pregnancy advances.

Since no published data was available on the histomorphological study of the placenta at different gestational periods, so, the result of the study could not be compared with such other studies. Few numbers of publications by other researchers with a similar study were available in other countries to compare with the findings of the present study. For the collection of second-trimester placenta foetal weight according to gestational age counted as a principal factor. In the present study, routine Haematoxylin & Eosin (H&E) stain and the light microscope were used.

Chorionic villi and placenta should be studied using Masson's trichrome, toluidine blue or periodic acid-Schiff (PAS) stains for improved visualisation. Further studies using ultrastructural and histochemical techniques are recommended. A similar study on gestational hypertension, gestational diabetes, anaemia, spontaneous abortion, and to compare with the present study is recommended.

CONCLUSION

In the present, study, it has been found that diffusion distance from foetal to maternal circulation found to be reduced with the advancement of pregnancy. So, it assumes that the exchange of gas and nutrition from foetal to maternal circulation increases according to the need of a growing foetus. Result of this study acts as baseline data from which we can compare histological changes of the normal placenta with diabetic, hypertensive, anaemic mother at different gestational periods in our population for future research.

CONFLICT OF INTEREST

The authors declare that they have no competing interests in publishing this article.

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