Journal of Rawalpindi Medical College (JRMC); 2008;12(2):52-56

Morphometry of Villous Membrane of Placenta at high and low Altitudes

Zarmina Saga*, Liaqat Ali Minhas**.

*Department of Anatomy, Rawalpindi Medical College, Rawalpindi.
** Department of Anatomy, Army Medical College, Rawalpindi

Abstract

Background: This cross sectional study was designed to compare the histomorphological characteristics of human placenta at low and high altitudes, with special reference to villous membrane. It was a cross sectional comparative study, conducted at Anatomy department of Army Medical College Rawalpindi, from October 2002 to April 2003.

Methods: Forty placentae were collected from normal cases at Rawalpindi (low altitude/ LA) and 40 were collected at Skardu (high altitude/HA). Samples of placental tissue were taken and processed. Histological study was done in H & E and PAS stained sections. Detailed morphology of terminal villi and villous membrane was studied. Thickness of villous membrane was measured which has not been mentioned in previous studies.

Results: Mean value of villous membrane thickness in LA and HA groups was 2.188 ± 0.023 μm and 2.027 ± 0.020 μm respectively. The quantitative difference between mean thickness of villous membrane in LA and HA groups was statistically significant

Conclusion: Thickness of villous membrane was significantly less in high altitude group probably showing adaptive changes to overcome the effect of reduced oxygen tension.

Keywords: High Altitude, terminal villi, villous membrane

Introduction

The placenta is an organ essential to the development of the fetus. In mammals, the fetus develops inside the body of the mother. The placenta is attached to the fetus by a cord containing blood vessels - the umbilical cord. The placenta is also firmly attached to the uterus of the mother. The role of the placenta is to bring the blood of the fetus close to the blood of the mother. This then allows oxygen and mineral salts to pass from the mother to the fetus, while carbon dioxide and other waste materials pass in the opposite direction. A variety of materials, including drugs and viruses, can pass across the placenta and enter the fetus. However, the mother provides some protection against infection.

The human placenta is of hemochorial type in which the maternal blood circulates in channels within the fetal syncytium. The fusion between fetal and maternal tissues is such that at birth placenta tears away as a unit.[1] This fetomaternal organ comprises of a fetal portion, the chorionic plate and a maternal portion, the decidual plate separated by a lacunar space, the intervillous space (IVS). A large number of finger like projections, "the villi", project into this lacunar space. These villi are comprised of a highly cellular core of mesenchyme invested by the trophoblast. The trophoblast is composed of outer syncytiotrophoblast and inner cytotrophoblast.[2]

The villi arising from the chorionic plate are the stem villi. Repeated branching of these stem villi into the IVS results in formation of branching villi. The branching villi further give rise to free or terminal villi, which are completely surrounded by maternal blood. These are the site of fetomaternal exchange.

As gestation continues, the terminal villi are reduced in diameter. The cytotrophoblast layer becomes inconspicuous since they contribute their cell mass to growing syncytium.[3] An uneven distribution within the syncytiotrophoblast results in clusters of nuclei, the syncytial knots, with intervening thin regions of cytoplasm. The fetal capillaries come in close apposition with these thin regions and even bulge the surface of syncytiotrophoblast forming vasculosyncytial membranes (VSM). This process of syncytialization continues till term.[4]

The structures interposed between maternal and fetal erythrocytes of human placenta include the trophoblastic epithelium and a composite of connective tissues comprising stroma, fetal capillary endothelium and plasma. Collectively the layers from the outer aspect of trophoblast to the luminal aspect of endothelium constitute "the villous membrane" which

is the barrier between maternal and fetal blood and on which placental transfer depends. Since the exchange of nutrients and gases between mother and fetus is mostly passive, the most influential properties of the villous membrane are its exchange surface area and effective diffusion distance.[5]

Placental growth occurs throughout all three trimesters and, in each, oxygen tension plays a key role. Uncomplicated pregnancy at high altitude can be considered as a miracle of nature since the human high-altitude fetus is subjected to the double insult of hypoxia; firstly due to lowered maternal arterial PO2 and secondly due to decreased uterine blood flow.[6]

In view of this background knowledge the current study was designed to compare the histomorphological characteristics of human placenta at high altitude (Skardu - 8500 ft) with those close to sea level (Rawalpindi – 1800 ft). Special emphasis was laid on estimation of thickness of villous membrane.

Materials and Methods

Placentae from normal full term pregnancies were collected randomly. Forty High altitude (HA) samples were collected from Obstetric Department of District Headquarters Hospital Skardu, while 40 low altitude (LA) samples were collected from department of Gynecology and Obstetrics of Military Hospital Rawalpindi. Placenta from those pregnant women were included who remained at the same altitude (either low or high) throughout the gestational period. Secondly placentae of normal healthy neonates having birth weight 2500 gm or more were included.

After delivery, placenta was placed in 10% Formol saline solution for 24 - 48 hours. Placenta was divided into two halves with the line of incision passing close to the attachment of umbilical cord. One half with the insertion of umbilical cord was taken and placed vertically on paper. A slice of about 1cm thickness was cut from this half of placenta. Three full depth samples of placental tissue, containing both maternal and fetal surfaces, were cut from this slice i.e. one from close to umbilical cord insertion (A), one from periphery (C) and one midway between A and C (B). One 5 mm thick complete circular section of umbilical cord was also taken (D).

Three full depth samples of placental tissue were further processed for paraffin sectioning. 4-5 μm thick sections were made and stained with two stains i.e, H&E and PAS.

Microscopic examination was carried out on terminal villi. They were recognized as smallest villi containing capillary loops and completely surrounded by blood. Complete circular cross sections were selected and villous membrane (Placental barrier) was identified.

Thickness of villous membrane was determined. This measurement was done in the regions of VSM i.e. syncytiotrophoblast and capillary endothelium without intervening nucleus and with minimal stroma. The thickness was determined in cross sections of five terminal villi per slide in A, B and C regions under 100X objective and mean was calculated. For this purpose ocular micrometer was used which was calibrated with standard stage micrometer in following manner;

The calibration of ocular micrometer was done with a stage micrometer. The stage micrometer is a microscopic slide with a 1-millimeter long scale etched on the surface. This 1-millimeter was divided into 100 divisions so that each division was equal to 0.01mm (10 μm). The stage micrometer was placed on the stage of a microscope and focused under 100X objective. The ocular micrometer or eyepiece reticule was placed in the eyepiece and aligned with the stage micrometer. The numbers of divisions of ocular micrometer corresponding with that of stage micrometer were noted and then the value of one division of ocular micrometer was calculated;

100 divisions of ocular micrometer =12 division of stage micrometer.

100 division of ocular micrometer =120 μm

Thus 01 division of ocular micrometer =1.2 μm.

The ocular micrometer scale was superimposed on the villous membrane at the area of VSM. Number of divisions from inner surface of capillary endothelium to the outer margin of cytoplasm of syncytial cell multiplied by 1.2 was taken as the actual thickness of villous membrane.

The data was fed in computer program SPSS.10 for Windows. The statistical significance of difference between the thickness of villous membrane in two groups was evaluated by using "Student's t-test". The difference was regarded significant if p value was less than 0.05.

Results

Histologically placenta was composed of two layered structures, chorionic plate and decidual plate, with an intervillous space (IVS). Chorionic plate was a layer of connective tissue containing blood vessels and lined by squamous cells. The decidual layer was composed of large polyhedral decidual cells with

eosinophilic cytoplasm and large vesicular nuclei. The IVS contained RBCs and cross sections of various villi. The smallest villi (the terminal villi) were seen lying free in the IVS. They were composed of a core of connective tissue, which was highly vascular. Two types of cells lined these villi. The outermost layer composed of syncytiotrophoblast, darkly staining cells without distinct cell membranes. On the inner aspect of these cells cytotrophoblast were present which were large cuboidal cells with rounded to oval nuclei. These cells were less basophilic as compared to the syncytiotrophoblast. They were occasionally present in cases of LA group but were more numerous in HA group.

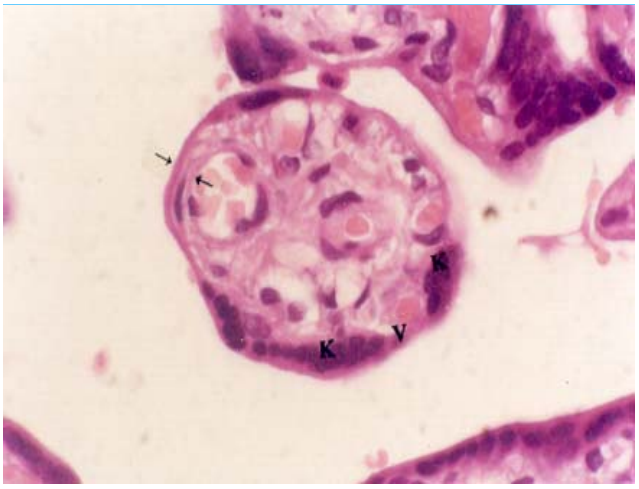

Fig 1. Photomicrograph showing terminal villus with syncytial knots (K), vasculosyncytial membranes (V) and villous membrane (→ ←). Case no.13-LA. H & E stain. Approx. 2400X.

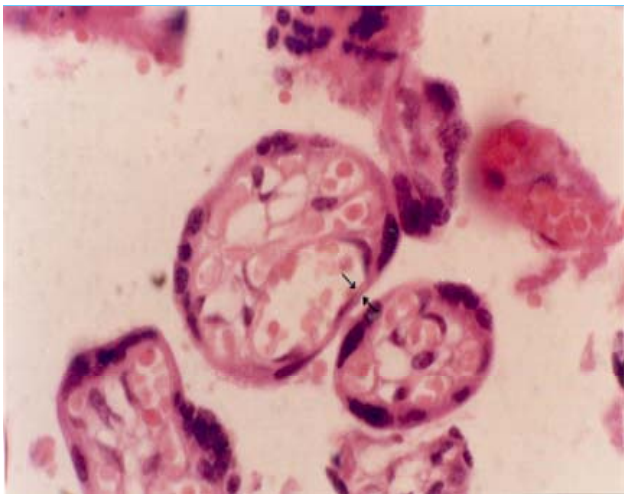

Fig 2. Photomicrograph showing terminal villus with villous membrane (→ ←). Case no. 30-HA.H & E stain. Approx 2400X.

The general architecture of the terminal villi was similar in both LA and HA groups. The core of the villus contained highly cellular stroma. At some places the nuclei of syncytiotrophoblast were clustered together forming syncytial knots. In this way thin regions of cytoplasm without nucleus were seen in-between forming VSM (Fig-1).

Villous membrane was made up of syncytiotrophoblast, villous connective tissue and capillary endothelium (Fig-1). At most of the places the stroma was missing thus the two other structures were joined together (Fig-2).

Thickness of the villous membrane was measured. Those areas were selected where capillaries were closely applied to the thin regions of syncytial layer i.e. VSM. The thickness was taken from the inner surface of endothelium to the outermost surface of syncytium. The measurement was done in five areas from each slide in A, B and C regions and mean was calculated.

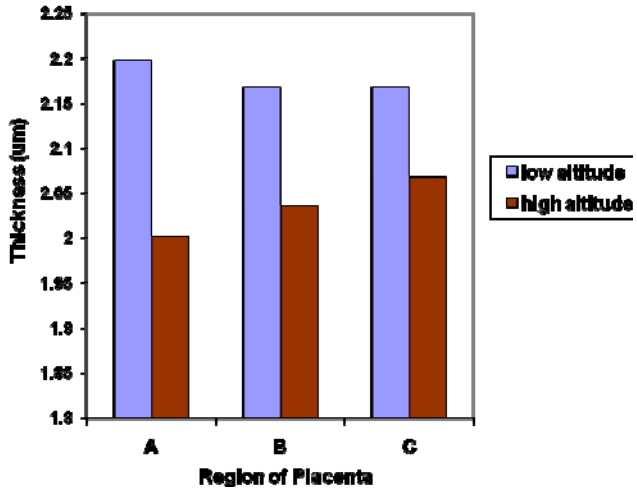

Fig 3. Mean thickness of villous membrane in different regions of placenta in LA and HA groups

In LA group the mean value of villous membrane thickness in all three regions was 2.198 + 0.024 μm, 2.168 + 0.020 μm and 2.168 + 0.025 μm while in HA group it was 2.002 + 0.029 μm, 2.036 + 0.023 μm and 2.068 + 0.033 μm respectively (Table-1, fig-3). The quantitative difference between mean thicknesses of

villous membrane in LA and HA group was statistically significant ($p<0.05$, Table-1).

On pooling the data from three regions the mean value of villous membrane thickness in LA and HA groups was 2.188 + 0.023 μm and 2.027 + 0.020 μm respectively (Table-2). The quantitative difference between mean thickness of villous membrane in LA and HA groups was statistically significant ($p<0.05$, Table-2).

Table-1
Mean thickness of Villous Membrane in La and Ha groups in different Regions of Placenta

Param-eters	Region of Placenta	LA Group Mean ± S.E. n = 40	HA Group Mean ± S.E. n = 40	Statistical significance between LA and HA groups
Thickness of villous membrane (μm)	A	2.198 ± 0.024	2.002 ± 0.029	P<0.05
	B	2.168 ± 0.020	2.036 ± 0.023	P<0.05
	C	2.168 ± 0.025	2.068 ± 0.033	P<0.05

Table – 2
Pooled Data of all three Regions Mean Thickness of Villous Membrane

Param-eters	LA Group Mean ± S.E. n = 40	HA Group Mean ± S.E. n = 40	Statistical Significance of difference between LA and HA groups
Thickness of villous membrane (μm)	2.188 ± 0.023	2.027 ± 0.020	P<0.05

Discussion

The intrauterine existence of fetus is dependent on one vital organ - "the placenta", which is required for maintaining pregnancy and promoting normal fetal development. It is sometimes described as the mirror of perinatal period as it shows many adaptations under different circumstances.

Throughout the world a large population permanently live at altitudes i.e. about 2500 meters above sea level.[7] The atmospheric pressure disturbances may complicate the high altitude pregnancies as fetus is subjected to the hypoxia. Chabes et al.[8] documented that the fetus is shielded from the hypoxic environment through certain placental mechanism, the most important being decrease in the diffusion distance between maternal and fetal circulation. The quantitative description of morphology by efficient and design-based methods have proved of great value for challenging earlier misconceptions and interpreting better the processes of growth, morphogenesis, adaptation, and functioning at the whole-organ level.[9] Human placental development involves co-ordinated angiogenesis and trophoblast outgrowth.[10]

In our study sections of placenta revealed that the villous membrane separated the maternal blood in IVS and fetal blood in villous capillaries thus acting as placental barrier. It was comprised of layers of trophoblast and fetal capillary with variable amount of connective tissue interposed. The membrane acts as physical barrier to transfer of respiratory gases and other metabolites. According to Boyd et al.[11] this villous membrane has a considerable mass accounting for roughly 12 % of total placental weight. They also documented that villous capillary dilatations take place towards the overlying syncytiotrophoblast and as more pressure is exerted, over a period of time, it may lead to remodeling of the latter thus forming vasculosyncytial membranes. The villous membrane at these places may thin out to as little as 1-2 μm. These thin specialized areas were previously called epithelial plates or "nephro-pneumoid regions" but the term "vasculosyncytial membrane" (VSM) was introduced and is still most widely used. These VSM are formed by the obtrusion of distended fetal capillaries into the trophoblastic layer and capillary wall is thinnest at the center of VSM, which indicates remodeling of endothelial cells at these sites.[12]

Thickness of villous membrane at VSM was found to be significantly less at HA. Several studies have supported this result.

Jackson et al.[13] demonstrated that at HA these VSM and syncytial knots decrease resistance to diffusion by 26 % as compared with that of a membrane with uniform thickness. They showed alteration in dimensions of villous membrane by using stereological methods and reported a decrease in arithmetic and harmonic mean thickness of VSM; the former proportional to tissue mass and oxygen consumption while the latter to resistance to gaseous diffusion. Mayhew et al.[14] demonstrated that in highland organ. diminished growth of villi was compensated by thinning of diffusion distances i.e. trophoblast and stroma. Other stereological studies showed decrease in villous domain size and a 4-fold increase in total intervillous volume at HA. Although villous domain decrease in size their number remain constant.[15]

Mayhew further supported that this thinning could serve to facilitate oxygen diffusion. It has been

proved that all functional capabilities of placenta (like transfer of nutrients from mother to fetus, removal of waste products from fetus and secretion of variety of hormones) depend upon a single cell, the syncytiotrophoblast. The syncytiotrophoblast layer constitutes the actual barrier for transplacental transport in human.[16]

The thinning of villous membrane component has not been found in organs of diabetic and smoker mothers most probably because of difference in origin of hypoxia. Studies by Rath et al.[17] reported thickening of trophoblastic basement membrane in passive smokers, which might contribute to fetal abnormalities, and growth retardation in this group. They defined VSM as the area of cytoplasm of syncytiotrophoblast in close approximation with the capillary, with minimal amount of stroma in-between.

Additional work by Zamudio[6] revealed that at HA harmonic mean thickness of villous membrane was reduced due to thinning of syncytiotrophoblast and location of fetal capillaries close to the membranes. This is possibly to compensate for the pre-placental type hypoxia to which the fetus is exposed. Normal vascular growth during implantation and placentation is critical for successful gestation and it is thought that vascular insufficiencies during placentation contribute to a number of obstetrical complications.[18]

Most of the findings correlate with the findings of previous workers. But there is no evidence in literature regarding actual estimation of villous membrane thickness. This estimation is difficult because of the problem of irregular appearance of the membrane due to natural variation in real thickness. In our study this point was considered and to avoid bias in estimation, measurement was done in areas of minimum thickness i.e. the VSM.

In conclusion, our study showed some quantitative differences in the structure of terminal villi from low and high altitude placental samples. Thickness of villous membrane at VSM was found to be significantly less at HA. It means that there is reduction in the diffusion distance between maternal and fetal circulation. This helps the mothers, living at high altitudes with reduced oxygen supply, to give birth to normal healthy individuals.

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