THE IMMUNOHISTOCHEMICAL AND ULTRASTRUCTURAL EVALUATION OF PERICYTES IN HUMAN FULL TERM PLACENTAS OF GESTASYONAL DIABETES MELLITUS

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ABSTRACT

Pericytes, vessel wall plays a stabilizing role in the regulation of blood flow in the microcirculation. The purpose of this study is based on non-diabetic pregnancies complicated by gestational diabetes pregnancies to investigate the morphological structure of pericyte cells. In this study, as a control group human placental tissues from normotensive pregnancies was collected from diabetic wome at 28-35 weeks of gestation. Pericytes with smooth alpha-actin positive cells, endothelial cells, and painted like a belt was surrounded. Pericytes, capillary plexus and endothelial cells of large vessels in the mesenchyme around the middle shows desmin positive reaction. Placental microvessels examined by transmission electron microscopy showed many pericytes. Placentas of gestational diabetes group, heterochromatin nucleus hypertrophy, dilatation of endoplasmic reticulum, mitochondria cristae in length, shortening was observed thickening of the filamentary structure. The contractile function of the barrier formed by endothelial cells, pericytes and can increase the contractions were considered

***Key words**: Gestational diabetes,pericyte cells,α smooth actin,Desmin*

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Introduction

Pericytes are multipotent mesenchymal-like cells found in association with small blood vessel walls. Pericytes are necessary components of microvasculature regulating development, stabilization, maturation, and remodeling[(1)].They are important for angiogenesis, the structural integrity of the microvasculature, and blood flow regulation. Pericyte loss or dysfunction is involved in numerous pathologies, such as hypertension, diabetic microangiopathy and tumor angiogenesis[(2,3,4)]. Pericytes on normal capillaries typically express desmin, but not α smooth muscle actin, whereas smooth muscle cells on arterioles and pericytes on venules were immunoreactive for both[(5)]and[(6)] Other reports have suggested that α smooth muscle actin may be considered a general marker for pericytes[(7,8)].

Gap junctions provide direct connections between the cytoplasm of pericytes and endothelial cells, and they enable the exchange of ions and small molecules.Desmin and alpha-smooth-muscle actin (α-SMA) are contractile filaments, and regulator of G protein signaling 5 (RGS-5) is a GTPase-activating protein; all three are intracellular proteins[(9)]. Alpha smooth-muscle actin is one of the six mammalian isoforms of the cytoskeletal protein actin. The beta and gamma nonmuscle actins are present in all cells, whereas α-SMA is normally restricted to cells of the smooth-muscle lineages. Certain nonmuscle cells have been shown to transiently express α-SMA, specifically fibroblasts, which are then referred to as myofibroblasts[(10)]. In this study, gestational diabetes morphometric structure of cells in the human placentas pericytes, endothelial cells, immunohistochemical and ultrastructural relationship between the assessment will be made.

Material and method

The study was performed on placentas from pregnancies monitored at the Department of

Obstetrics and Gynaecology of Medicine Faculty in Dicle University. Local ethics committee approval and written patient consent were obtained. In addition, as a control group, human placental tissues from 20 normotensive pregnancies was collected from diabetic women at 28-35, (20 placentas) weeks of gestation.Following diagnosis, all patients began home blood glucose monitoring while undertaking an appropriate diet. The level of glycemic control achieved by the patient was assessed by maternal glycosylated hemoglobin(HbA1c).

The placentas from women with gestational diabetes and from the control group were obtained. Immediately after delivery, normal and diabetic placenta were transported from the delivery room to the laboratory and, after preliminary gross examination, two series of tissue samples were obtained. The specimens were immersed in 10% buffered formaldehyde. Then, sections of 5 μm in thickness were cut and made into slides.

Immunohistochemistry

The immunohistochemical detection of alpha-smooth-muscle actin and desmin was performed as follows. After deparaffinization and antigen retrieval in a microwave oven, the endogenous peroxidase activity and the non-specific antigen binding sites were blocked. Following incubation with primary antibody (mouse monoclonal anti-human alpha-smooth-muscle actin; Santa-Cruz) 1:100, and secondary mouse monoclonal anti-human desmin (Dako) 1:100, respectively, for 40 min at room temperature, the detection was performed using DAB + peroxidase kit (Dako). Sections were counterstained with hematoxylin. Simultaneous control experiments with the omission of either primary or secondary antibody gave negative results.

Electron Microscopy technique

The pieces of tissue were immediately placed in 2,5% glutaraldehyde,buffered for 4h, then fixed in OsO4 for 2h, dehydrared in graded ethanols,and embeded in araldite.Semithin sections of 1μm thick were cut and stained with methylene blue-azure II for light microscopic examination Thin sections of 70nm thick were stained with lead citrate-uranyl acetate and examined and photographed under Karl Zeiss Evo LS10 Electron microscope

Data are presented as means with standard deviations or as percentages. The significance of the difference between groups was calculated with twotailed Student's t-tests for independent samples.

Result

We defined the different histologic abnormalities clinical significance and diabetic placentas women from time to time control 20 pieces of gestational diabetes and normotensive of pericytes cells diameters located in the chorionic villus cells and pericytes were measured ($p<0.005$) Gestational diabetic group, chorionic villi and in the endothelial cells of blood vessels under the pericytes cell expression were examined. Pericytes with smooth alpha-actin positive cells, endothelial cells and painted like a belt was surrounded. The vessel shows a relatively normal and uniform layer of αSMA intimately associated with luminal endothelial cells. (Figure-1) pericytes, capillary plexus and endothelial cells of large vessels in the mesenchyme around the middle shows desmin positive reaction. α smooth actin and desmin expression, compared with the vessel wall desmin staining was found to be more intense. Chorionic capillaries and large vessels, the surrounding mesenchymal tissue, desmin expression was intense(Figure-2)

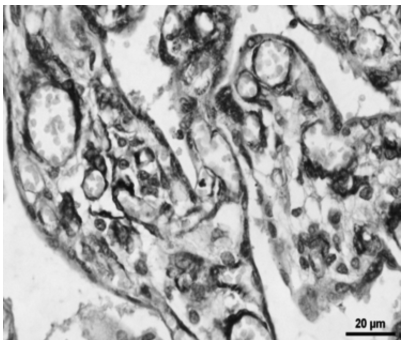

Figure 1-α smooth actin positive reaction in pericyte cells (Arrow).

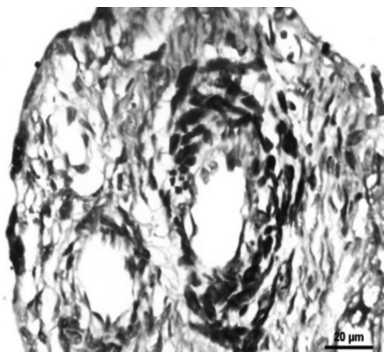

Figure 2: Note an increased expression of desmin in the capillary)plexus inside the chorion and in the large vessels in the intermediate mesenchme (Arrows).

Ultrastructural examination of placental non-diabetic group;The pericyte nucleus is round and the endothelial nucleus is elongated and flat shaped.The nucleus of the pericyte is a heterochromatin that their cytoplasm is electron-dense and contains fewer microfilaments (Figure-3). Placentas of gestational diabetes group, heterochromatin nucleus hypertrophy, dilatation of endoplasmic reticulum, mitochondria cristae in length, shortening was observed thickening of the filamentary structure (Figure-4). Endothelial cells and pericytes share a common basement membrane. Diabetic group showed dilation of the interval between the basement membrane cells and pericytes.

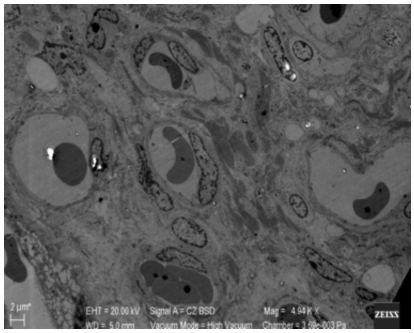

Figure 3: The pericyte nucleus (thick arrow) is round and the endothelial nucleus is elongated and flat shaped (thin arrow) cytoplasm containing electron-dense and less microfilaments heterochromatin (Uranyl-acetate-6800).

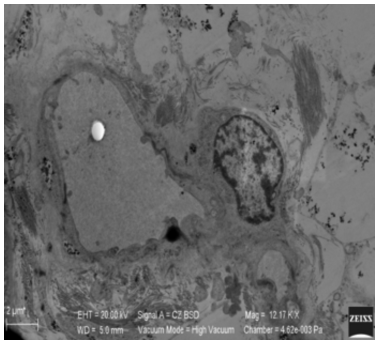

Figure 4: Hypertrophy in pericyte cells (arrow), thickening of the filamentary structure (star) (Uranyl-acetate-7200).

Discussion

Pericytes are located in a sub-endothelial location in arteries and arterioles throughout the adult vascular area ,microvascular endothelial function of the placenta, pregnancy is a key to continue on a regular basis. Pericytes, determine the characteristics of the microvascular bed microvascular basement membrane and also helps to protect. vascular smooth muscle cells associated with the installs[1,3]. Microvascular endothelial cells, pericytes to support the contractile effect to protect the integrity and permeability-enhancing effect. Pericytes are local regulatory cells that are important for the maintenance of vascular homeostasis

In diabetic pregnancies complicated by fetal growth retardation syncytial knots are found more frequently, the percentage of vasculo-syncytial membranes tends to be lower, and the trophoblastic basement membrane is significantly thicker[11]. Aland Lawrenson[12] emphasized the interaction of pericytes and endothelial cells and its importance for maturation, remodelling and maintenance of the vascular system via the secretion of growth factors, modulation of extracellular matrix, and regulation of vascular permeability. Most investigators have used antibody directed against alpha smooth muscle actin (αSMA) to identify pericytes. While pericytes are clearly capable of expressing αSMA, the expression of this protein in vivo may be associated with functional heterogeneity within the capillary and in vitro may be a marker of differentiation. In their capillary location, most pericytes are αSMA negative[13,14,15]. In our study group, pericytes cells, expression of alpha-smooth significantly observed. αSMA well characterized and can be considered to be one of the most commonly used markers pericyte.

During pregnancy, the placenta complications occurring microvascular endothelial cells is a key task for the protection of the status of angiogenesis.In our study, the contractile function of the barrier formed by endothelial cells, pericytes and can increase the contractions were considered.

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