

Placental expression of vimentin, desmin and ultrastructural changes in the villi in patients with HELLP syndrome

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Abstract. – OBJECTIVES: To examine placental expression of vimentin and desmin in relation to ultrastructural changes within the placental villi in cases of HELLP syndrome.

STUDY DESIGN: Formaldehyde-fixed and paraffin-embedded specimens of 15 healthy pregnant and 13 Hemolysis, elevated liver enzymes, and low platelet count (HELLP) syndrome, placentas were used for Harris hematoxylin staining, vimentin and desmin immunohistochemistry, and transmission electron microscopy (TEM).

RESULTS: Increased of fibrinoid necrosis in vascular wall and the periphery of villi were observed in sections of the placentas with HELLP syndrome. Increased expression of vimentin in the intervillous area and increased expression of desmin on blood vessel wall, were seen in placentas of patients with HELLP syndrome when compared to placentas of healthy pregnant.

CONCLUSIONS: Augmentation of intermediate filaments, desmin, vimentin may disturb normal movements of endothelial cells, and may display placental dysfunction that is unable to compensate the endothelial instability and the related hypertension in HELLP syndrome. Further studies are needed to get more definite results and also to compare HELLP syndrome with preeclampsia.

Key Words:

HELLP Syndrome, Placental expression, Vimentin, Desmin.

drome is categorized as a gestational hypertensive disorder, and seen as the more severe variant of preeclampsia (PE)^{1,2}.

Hemolysis, elevated liver enzymes, and low platelet count (HELLP) occur in 20% of severe PE cases. The pathogenesis of HELLP syndrome is not clear and the obstetric approach with the induction of delivery is still the only specific therapy in HELLP syndrome. Involvement of the coagulation system is seen in HELLP patients, which is not present in PE patients without HELLP³. Angiogenic factors, cell adhesion proteins, immunological factors, matrix metalloproteinases and their inhibitors are all anticipated to play a role in this crucial process of spiral artery dilatation^{4,5}.

In order to characterise the differentiation of placental stromal cells in the human placenta, a variable layer of extravascular stromal cells lying beneath the trophoblast expressed vimentin (V) or vimentin and desmin (VD) were investigated on placental tissue of different gestational age. The expression patterns of the cytoskeletal proteins vimentin, desmin, alpha- and gamma-smooth muscle actin, pan-actin, smooth muscle myosin, and the monoclonal antibody GB 42, as a marker of myofibroblasts and intermediate filaments were arranged as concentric layers with increasing stage of differentiation around the fetal stem vessels in placental villi⁶. The placenta and the incomplete trophoblast invasion of spiral arteries have a central role in pathogenesis of HELLP syndrome, but especially in severe preeclampsia and in the HELLP syndrome there is a systemic endothelial activation and damage⁷.

The aim of this study is to examine placental expression of vimentin and desmin in relation to ultrastructural changes within the placental villi in cases of HELLP syndrome.

Introduction

HELLP syndrome is a form of preeclampsia during pregnancy that often progresses to a life-threatening complication. HELLP usually occurs in the later stages of pregnancy, or sometimes after delivery. Despite reports describing HELLP in patients with normal or minimally elevated blood pressure without proteinuria, HELLP syn-

Materials and Methods

This study was performed at the Dicle University, School of Medicine, Department of Obstetrics and Gynecology. The study included 13 pregnant patients with HELLP syndrome (study group) and 15 healthy pregnant at between 24 and 35 weeks gestational age (control group). The study was approved by the local institutional review board. Control group, healthy pregnancy was defined as pregnancy characterized by normal blood pressure values ($< 140/90$ mmHg), negative proteinuria and normal values of laboratory findings. The HELLP syndrome was defined as hemolysis (peripheral blood smear findings and lactate dehydrogenase (LDH) > 600 U/L, or serum total bilirubin level > 1.2 mg/dL), decreased platelet count ($< 100,000$ cells/L), and elevated liver enzymes [aspartate aminotransferase (AST) > 70 U/L].

Gestational weeks of the women were determined according to the last date of menstruation and/or ultrasonographic measurements. All patients included in the study were monitored in the intensive care unit. In HELLP Group vital signs of all patients were monitored and magnesium therapy was initiated. Loading dose of magnesium (6 g) was administered in 20 minutes, and maintenance dose was given at a rate of 2 g per hour.

On the first admission, variables such as blood pressure, whole blood counts, ALT, AST, and LDH were evaluated. The decision to deliver was determined based on obstetric anamnesis, maternal and fetal status, and Bishop Scores. Vaginal delivery was preferred, however in the presence of unsuitable cervix, fetal distress, and in patients with previous caesarian delivery, Caesarian section was performed.

Immediately after delivery, normal and pathological placentas 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, and 5 μ m thick sections were cut and made into slides. These were processed for Hematoxylen-Eosin and Masson's trichrome staining, carried out according to conventional procedures.

Immunohistochemistry

The immunohistochemical detection of vimentin 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 vimentin; Santa-Cruz, Santa Cruz, CA, USA) 1:20, and secondary mouse monoclonal anti-human desmin (Dako, Troy, MI, USA) 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.

Transmission Electron Microscopy

The tissue samples were immediately placed in 2.5% glutaraldehyde, buffered for 4 h, then fixed in OsO_4 for 2 h, dehydrated in graded ethanols, and embedded in araldite. Semi-thin 1 μ m-thick sections were cut and stained with methylene blue-azure II for light microscopic examination. Thin sections of 70 nm were stained with lead citrate-uranyl acetate, and examined and photographed under KARL Zeiss EVO LS10 STEM Electron microscope (Perbody, MA, USA).

Statistical Analysis

Statistical analyses were carried out using the statistical package SPSS 15.0 for Windows (SPSS Inc., Chicago, IL, USA). The Mann-Whitney U test was performed to compare data from control and HELLP patients.

Results

The characteristics of the patients studied are summarized in Table I. Histopathological comparison was made of placental morphology between the two groups. Structural differences were found between the normal placental terminal villi (Figure 1 a) and pathological forms of villi were observed in HELLP placenta.

Syncytial nodes and an increase in syncytial bridges, heavy bleeding foci were seen in the intervillous space of the histopathological findings in the HELLP group. Chorionic villous maturation, vascular dilatation and progression of villous congestion were also increased in the connective tissue (Figure 1 b,c). In another section obtained from the HELLP group, with the expansion of blood vessels, fibrinoid necrosis becomes evident in the arterial wall, and fibrous tissue was increased in stromal cells and connective tissue fibers. Mesenchymal cells are loosely arranged throughout the villous interior, and contain the intermediate filament (IF) proteins vimentin and desmin.

Table 1. Characteristics of control and HELLP groups.

Characteristic	Controls (n = 15)	HELLP (n = 13)	p value
Age (year)	28.0 ± 4.0	29.9 ± 2.5	NS
Parity	1.8 ± 6.7	1.7 ± 7.2	NS
Gestational age at delivery (weeks)	31.2 ± 2.2	30.1 ± 1.7	NS
Systolic blood pressure at registration (mmHg)	117.0 ± 10.3	156.1 ± 12.6	< .001
Diastolic blood pressure at registration (mmHg)	70.3 ± 8.1	91.5 ± 13.4	< .001
Placental weight (g)	432.6 ± 48.4	380.0 ± 22.7	< .001

Endothelial damage has caused extravasation of blood components and the formation of fibrin in chorionic vessel wall as a result of shrinkage due to degenerative changes in endothelial cells in placentas with HELLP syndrome (Figure 1 b). Tunica intima injury and endothelial dysfunction has been observed in a majority of chorionic villi vessels. Increase of intravillous nonvascular connective tissue fibers and desmin, vimentin cytokeratin protein expression around blood vessels has been concentrated at the location of the vessel wall damage in other sections of chorionic villi, in placentas with HELLP syndrome (Figure 1 d). Fusiform-shaped view showed degenerative changes in endothelial cells and also thinning of the endothelial basement membrane increased distribution of blood cells and fibrin formation in assessment of the electron microscope (Figure 2 c).

Large vessels of the chorionic villi and stem villi showed thick walls consisting of vimentin, with

desmin-positive cells in cases with HELLP syndrome. The HELLP group showed positive expression of vimentin around blood vessels, connective tissue, and stromal cells (Figure 1 d,e).

The immunohistochemical detection of IF desmin demonstrated noticeable differences between normal and HELLP placentas villi (Figure 1 f,g). Desmin was confined to the bodies and cytoplasmic projections of stromal cells, which had a close relationship to arteries. In transverse sections, desmin-positive cells were apposed to the outer surface of arteries; their desmin-positive cytoplasmic projections were spread to the subtrophoblastic villous area, where arteries were mainly located. In sections from the HELLP group, smooth muscle cells showed intense expression of desmin. The increased of fibrinoid necrosis in vascular wall and the periphery of villi were observed in sections of the placentas with patients of HELLP syndrome (Figure 1 b,c). In-

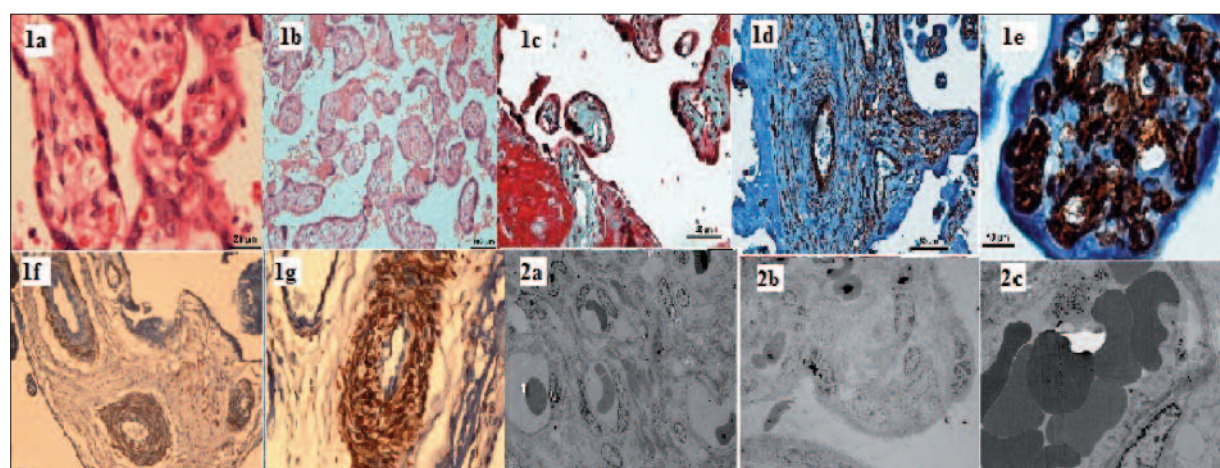


Figure 1. Control group H-E Bar 20 µm. **1b**, HELLP group: Sincistal nodes and an increase in syncytial bridges H-E Bar 100 µm. **1c**, HELLP group: Fibrinoid necrosis and a increase fibrous tissue (Trichrom-Masson Bar) 50 µm. **1d**, HELLP group: Vimentin expression in stem villous (Brown-black) Bar 50 µm. **1e**, HELLP group: Around blood vessels, connective tissue, increased expression of vimentin Bar 10 µm. **1f**, HELLP group: Desmin expression in choronic villous Bar 100 µm. **1g**, HELLP group: Desmin positive expression in smooth muscle cells Bar 50 µm. **2a**, Control group (Uranyl-acetate X6800). **2b**, HELLP group: Syncytial degeneration, reduction of microvilli (Uranyl-acetate X8600). **2c**, HELLP group: An increase in collagen and hypertrophy in pericyte cells vacuolation was seen in some places (Uranyl-acetate X6800).

creased expression of vimentin in the intervillous area and increased expression of desmin on blood vessel wall, supports the pathological findings by light microscopy in patients with HELLP syndrome (Figure 1 e,g). Ultrastructural examination, syncytial degeneration, and reduction of microvilli vacuolation were seen in some places. In Figure 2 a is healthy placenta and we observed an increase in the collagen component of villous stroma (Figure 2 b) in cases with HELLP syndrome. Degenerative changes of heterochromatine large nuclei in syncytial bridges as a result of junction of the syncytiotrophoblast cytoplasmic cells and cisternal expansion with an increased vacuoles in endoplasmic reticulum were observed in ultrastructural section of the HELLP group placentas. Edema located in the sub syncytial area due to syncytial cells changes, and also loss of the cristae in mitochondria of cytotrophoblast cells were observed (Figure 2 b).

Another section from the HELLP group showed collagen fibers, pericytes hypertrophy and increase in villus (Figure 2 c). In addition, there was thinning of the endothelial cell membrane, which showed deterioration in places. Platelets was observed in blood vessels that showed the electron dense (Figure 2 c).

Discussion

HELLP syndrome is a systemic obstetric complication and pathogenesis is not completely understood. Fischer et al⁸ investigated vascular reactivity in both PE and HELLP patients, and showed that vascular resistance during reactive hyperemia was increased in women with PE compared with both the HELLP group and control subjects. They concluded that vasodilatory reactivity is reduced in PE but not in HELLP syndrome, suggesting different pathogenetic mechanisms in the two populations, and probably a more pathological vascular development in PE than in HELLP. Augmentation of cytoskeletons in fetal capillary endothelial cells is one of the well known pathologic changes in toxemic placentae. There are few studies on these filaments, especially on their ontogeny.

Vimentin and desmin are intermediate filament proteins found in various mesenchymal and skeletal muscle cells, respectively⁹. Different subpopulations of extravascular stromal cells were distinguished according to typical co-expression patterns of cytoskeletal proteins. Around

the fetal stem vessels in term placental villi they were arranged as concentric layers with increasing stage of differentiation. A variable layer of extravascular stromal cells lying beneath the trophoblast expressed vimentin or vimentin and desmin. They were mitotically active. Significant expression of intermediate filament proteins, desmin and vimentin, in the inter-villous area of connective tissue and vessel wall were shown that cytokeratin mechanism may play an active role in HELLP syndrome. Desmin and vimentin thought to play a role in villous contractility and modulating by impact on maternal and fetal placental circulation. Ultrastructurally, V cells resembled typical mesenchymal cells and VD cells corresponded to fibroblasts⁶. In our study we showed positive and strongly increased immunoreactivity of vimentin in vessel lumen and the stromal cells around the vessel wall.

Vinnars et al. showed that in addition to retroplacental hematoma, placental infarction and decidual arteriopathy are also significantly more frequent in preeclampsia and HELLP syndrome¹⁰. Some researchers reported that desmin occurs in different forms with the commencement of the development of decidua. This is due to increased synthesis of cell shape and organelle distribution of activity associated with rapid transformation¹⁰⁻¹³. In the present work we showed vimentin and desmin expression in the stromal blood vessels and connective tissue areas of the region in the tunica media layer, combined with increased mesenchymal cell density in placentas with HELLP syndrome.

The basis of the pathophysiology of preeclampsia and HELLP syndrome is vasospasm. Hemolysis is one of the major features of HELLP syndrome. Red blood cell fragmentation caused by more blood flow formed along the damaged endothel, leads to tunica intima injury, endothelial dysfunction and the formation of fibrin deposition. Vascular hemorrhage and fibrin deposition changes due to endothelial damage was observed significantly in HELLP syndrome also in our study. Increased fibrin deposition and the distribution of the free form of the blood compounds in placental sections, supports the pathophysiological involvement of HELLP syndrome. Subendotelial fibrin storage takes place in the vessel wall. This result was significantly observed by us.

Degenerative changes of heterochromatine large nuclei in syncytial bridges as a result of junction of the syncytiotrophoblast cytoplasmic

cells and cisternal expansion with an increased vacuoles in endoplasmic reticulum is defined as a sign of moving towards to cell apoptosis (Figure 2 b). The degenerative change in the area of intravillous syncytial nodes, subsyncytial edema, increase of collagen fiber and loss of mitochondrial cristae in sitotrofoblast; induce apoptosis as well as significantly induced endothelial damage were observed.

Increase of connective tissue fibers and protein expression of vimentin, desmin around blood vessels especially concentrate at the location of the damage vessel wall for the repair of vessel wall and the fluidity of the blood. This report can be support by advanced techniques such as western blot, immune fluorescent assay in future studies.

Conclusions

We observed that increased expression of vimentin in the intravillous area and increased expression of desmin on blood vessel wall in patients with HELLP syndrome when compared to placentas of healthy pregnant. Augmentation of intermediate filaments, desmin, vimentin may disturb normal movements of endothelial cells, and may display placental dysfunction that is unable to compensate the endothelial instability and the related hypertension in HELLP syndrome. Further studies are needed to compare HELLP syndrome with preeclampsia.

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