HISTOLOGICAL MODIFICATIONS OF THE FETO - PLACENTAL INTERFACE IN PREGNANCY INDUCED HYPERTENSION

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Abstract

Objective. To present the main structural modifications of the feto-placental interface, in pregnancy induced hypertension (PIH).

Material and Method. We have studied the main microscopical modifications of 68 placentas obtained after delivery for two equal groups: one group (**N1**=34), representing mothers with PIH and another group (**N2** = 34) normotensive mothers. The samples obtained by sections were specificaly prepared for the study of 3 types of histological stains and 2 types of immunohistochemical stains. For the histological examination we used optical microscopy for observing mainly the lumen of spiral arteriole and changes in its tunica intima and media.

Results. We registered the following structural modifications in the pregnancies with PIH versus normal ones: changes in endothelium –76,47%, fibrinoid necrosis – 73,52%, the hypertrophy of tunica media – 67,64%, bridging syncytial knots –32,35%, avascular small terminal villi with hyaline fibrosis of the stroma–41,17% and the thrombosis of the spiral arterioles –26,47%.

Conclusions. A better understanding of the immunohistological damages demonstrated through our study, concerning the preeclamtic feto-maternal interface, will change in the future, our understanding about the role of this placental unit in PIH.

Key words: Pregnancy Induced Hypertension (PIH); Fetal Chronic Hypoxemia; Feto-maternal interface; Histological modifications.

Introduction

Preeclampsia is a major problem of modern obstetrics and various studies are mentioning PIH as a severe complication, one of the largest causes of maternal and perinatal morbidity and mortality of about 5 – 7% pregnancies throughout the world[1,2]. The gravity of the disease is a real emergency in obstetrical departments. The incontestable paradox is the fact that after the birth and the delivery of the placenta, the arterial hypertension disappears[3]. Because PIH is associated with the increase of the feto-placental vascular resistance[4], the disease represents one of the most important causes of: intrauterine growth limitation, premature birth, low birth weight, perinatal mortality.

Material and method

The study was performed with the written consent of the mothers between january 2008 and december 2010. We have studied the main microscopical modifications of the 64 placentas - obtained after delivery - from the two equal groups: one group (**N1**=34), representing mothers with (PIH) and another group (**N2** = 34), with normotensive mothers.

The main factor of differentiation was the value of the blood presure: For the group of normotensive pregnant women, the values of the systolic TA ranged between 100-135 mmHg, and of the diastolic TA, between 60-85 mmHg. The difference to the hypertensive pregnant women group was made only in the cases where the values of the systolic TA > 140mmHg and diastolic TA > 90 mmHg.

Significant differences in the rest of the clinical parameters between the two groups were registered also for the gestational age, birth weight, type of the birth and immediate neonatal adaptation.

For both groups, the following cases were not included: those with essential hypertension, multiple pregnancy, diabetes mellitus, chronic renal diseases, epilepsy and hematological disorders.

Specimens

Samples were obtained from the 64 placentas immediately after the birth.We have taken at least 2 samples of sections made from both - maternal and fetal - side of the placenta, for the study of 3 types of histological stains and 2 types of immunohistochemical stains.

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For the histological examination of the samples we used optical microscopy for observing mainly the lumen of spiral arteriole and changes in its tunica intima and media. They were in fixation in 4% buffered formalin, for 24-48 hours.

Hematoxylin–Eosin technique
- fixation in a 10% formalin solution
- dehydration in ethanol gradated series
- sedimentation in xylene
- sections
- paraffining
- deparaffining
- hydration and coloring with hematoxylene – eosine

Van Gieson's technique
- tissue sections to Ethyl Alcohol and stain with Weigert's iron hematoxylin for 15 minutes
- wash in running water for 15 minutes and rinse with distilled water
- Van Gieson's stain for 5 minutes and rinse in distilled water
- rinse rapidly in 70% Ethyl Alcohol
- dehydrate rapidly in Absolute Alcohol
- clear and mount in Neutral balsam

Masson's Trichrome technique
- deparaffinize and hydrate to distilled water
- slides in 40 ml of Bouin's solution contained in a plastic coplin jar and microwave
- mix solution with beral pipet
- incubate slides in heated Bouin's solution for 15 minutes in a fume hood
- wash slides in tap water until sections are clear
- stain in working Weigert's hematoxylin 5 minutes
- wash slides thoroughly in tap water
- 0.5% Hydrochloric acid alcohol for 5 seconds
- wash in running tap water for 30 seconds and rinse in two changes of distilled water
- stain in TRICHROME solution for 15 minutes and wash slides in tap water
- rinse in 0.5% Acetic acid 10 seconds and in distilled water
- dehydrate through graded alcohols
- mount with resinous mounting media

Immunohistochemistry

The IHC experiment was performed using the DAKO LSAB2 System method.

Samples sections were rehydrated, washed and then rinsed in PBS (pH 7.2). Antigen retrieval was achieved by using the HIER (Heat Induced Epitope Retrieval) method. IHC staining was performed upon 0.5 μm thick, on PolysineTM slides wich were incubated with 3% hydrogen peroxide solution for 5 minutes, then washed with PBS. Formalin-fixed, paraffin-embedded tissues were incubated so the slides could react with a labelled avidin-biotin complex, peroxidase-labelling detection system (Vector Universal Elite kit) and then treated with 3,3'-diaminobenzidine-peroxidase substrate solution, as chromogen (DAB Tablets, S3000-Dakopatts, Glostrup Denmark) until color was visualized. It was done using the method EmVision Dual Link-HRP. All reagents and supplies for the technique were from Dako, Denmark.

The primary antibodies were mouse monoclonal anti-Human Cytokeratin (code M 0821 Dako, 1:50) and monoclonal mouse anti-Human CD34 (Class II, clone QBEnd-10, DAKO, 1:50). Those were incubated for 30 minutes. The negative control reagent used for LSAB2 was Universal Negative Control, Rabbit (code N1699) and Dako Mouse IgG1 (code X0931) diluted to the same mouse IgG concentration as the primary antibody. Sections were washed twice in distilled water to stop the reaction, then counterstained in hematoxylin, washed, dehydrated, cleared in xylene, mounted with DPX, and glass cover-slipped.

Sections were examined with a ×100 objective on a AmScope microscope, and images were captured using a High speed 1.3 Megapixel USB 2.0 digital camera AmScope and a DN–100 digital imaging system.

Results

Histological aspects of the placentas comparing group N1 with group N2, we have registered the following modifications – Tabel 1:

- ***changes in endothelium*** were - enlargement, atrophy, disruption
- the considered pathognomonic lesion - the ***fibrinoid necrosis*** wich affects ***the wall of the spiral arteriole***
- the ***hypertrophy of the smooth muscles tunica media***
- ***avascular small villi*** meaning ***terminal villi*** showing the total loss of villous capillaries and bland ***hyaline fibrosis of the villous stroma***
- fibrin and/or bridging **syncytial knots** and **villous agglutination** is seen as clusters of adherent distal villi agglutinated
- the **thrombosis of the spiral arterioles**

In the group N2, by the microscopical examination, villous structures appeared almost normal, the connective tissues of each villous covered by trophoblastic cell layers and rich in fetal capillaries. The intervillous spaces were filled with maternal blood separated from each other. Figure 1.

In the group N1, microscopical changes of the placentas showed diffuse hypoxia histologically diagnosed based on the next main modification:
- heterogenous placental maturation – Figures 2 and 3,
- decreased chorionic villi by amount of the extracellular matrix,
- decreased density of the villous cytotrophoblastic cells and Hofbauer cells of the villous
branching of capillaries excessive,
- fetal capillaries have usually disappeared in most villi – Figure 4,
- syncytial knotting (smudgy and granular nuclear chromatin).

Tabel 1 – Histolological modifications of the placentas in the 2 study group.

Nr. crt	Structural modifications	P I H + (N1 group)		P I H – (N2group)	
		Nr.	%	Nr.	%
1.	Endothelium - enlarged, disrupted, atrophied	26	74,47	3	8,82
2.	Fibrinoid necrosis of the wall of the spiral arteriole	25	73,52	1	2,94
3.	Hypertrophy of the smooth muscles tunica	23	67,64	2	5,88
4.	Hyaline fibrosis of the villous stroma and avascular small terminal villi	9	26,47	0	0
5.	Bridging syncytial knots and fibrinous distal villous agglutination	11	32,35	0	0
6.	Thrombosis of the spiral arterioles	14	41,17	0	0

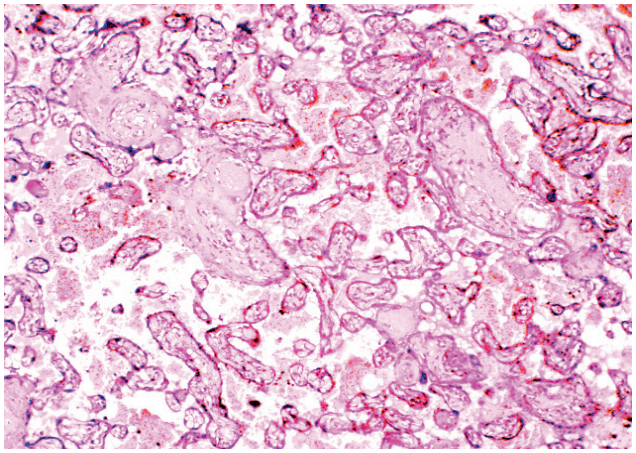

Figure 1 – Villous covered by trophoblastic cell layers and rich in fetal capillaries (H.E., x 40).

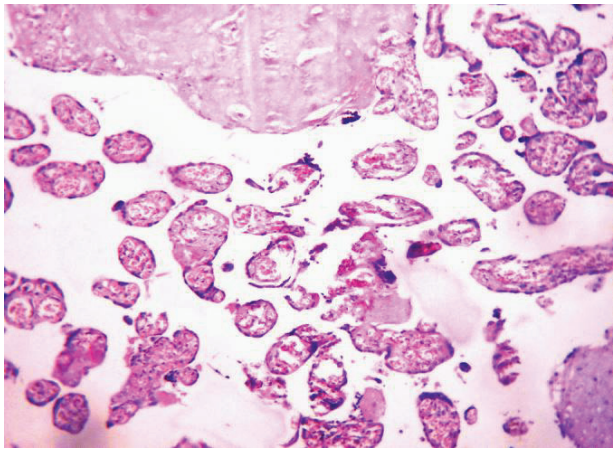

Figure 2 - Avascular terminal villi total loss of villous capillaries (H.E., x 40).

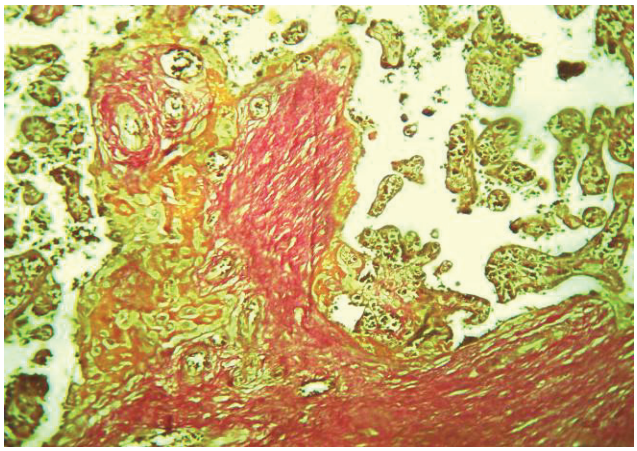

Figure 3 – Heterogenous villous maturation, hyaline stromal fibrosis (Van Gieson's x 40).

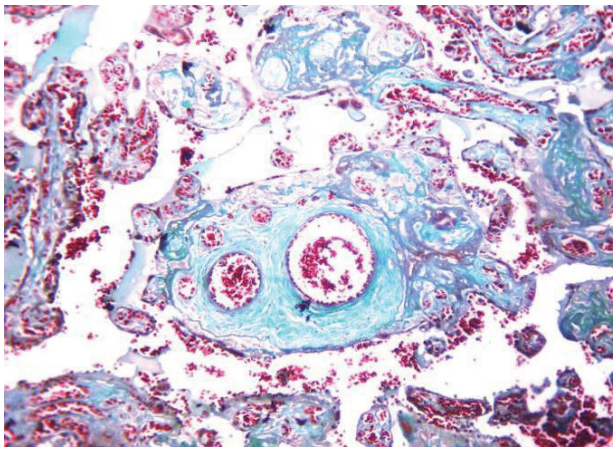

Figure 4 – Acute atherosis of the arterial smooth muscle (Masson's Trichrome, x 40).

The decidua presented some chorionic pseudocysts, laminar fibrinoid necrosis of the arterial smooth muscle with acute atherosis, which is characterized by and a lot of arterioles showed endothelial degeneration with progressive fibrosis and obliteration – Figures 5, 6 and 7.

The nuclei of the syncytiotrophoblasts had the tendency to develop clusters and sprouts protruding into the intervillous spaces – Figure 8.

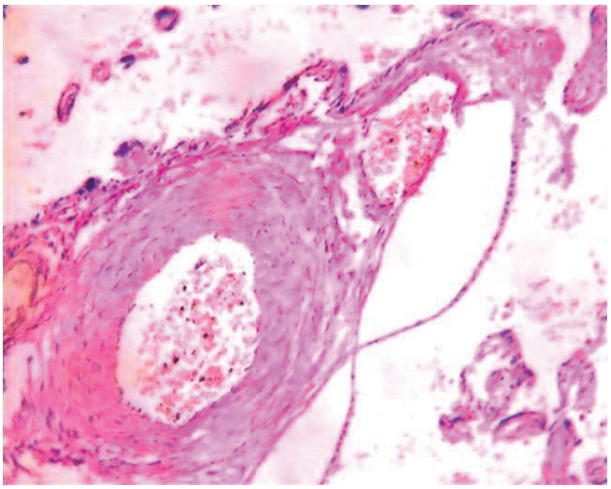

Figure 5 – Decidual chorionic pseudocysts, with laminar fibrinoid necrosis (H.E., x 100).

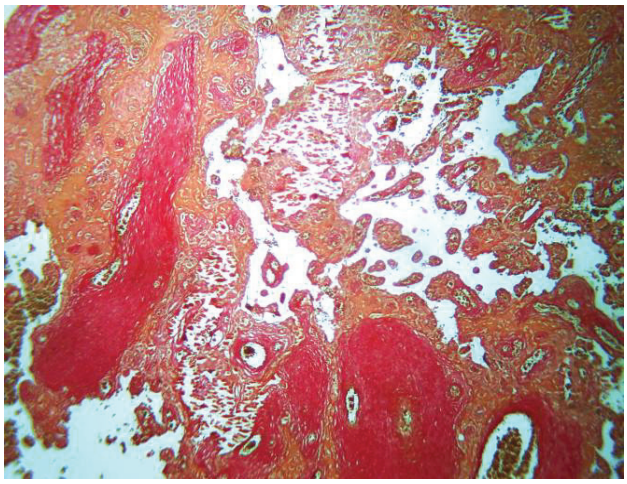

Figure 6 – Endothelial degeneration, fibrosis, obliteration of arterioles (Van Gieson's x 40).

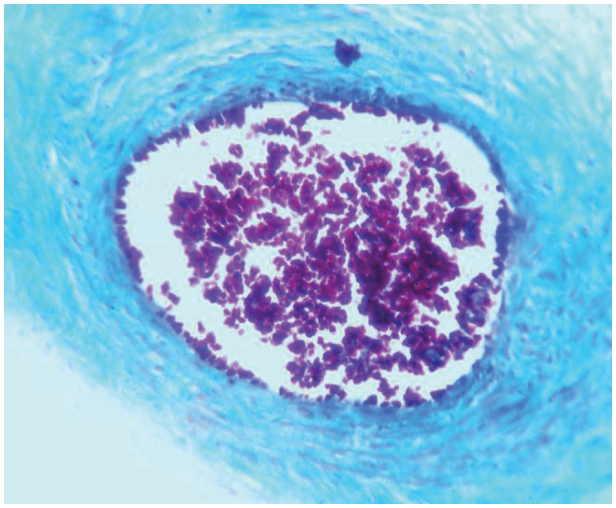

Figure 7 – Fibrinoid necrosis of the arterial smooth muscle (Masson's Trichrome, x 100).

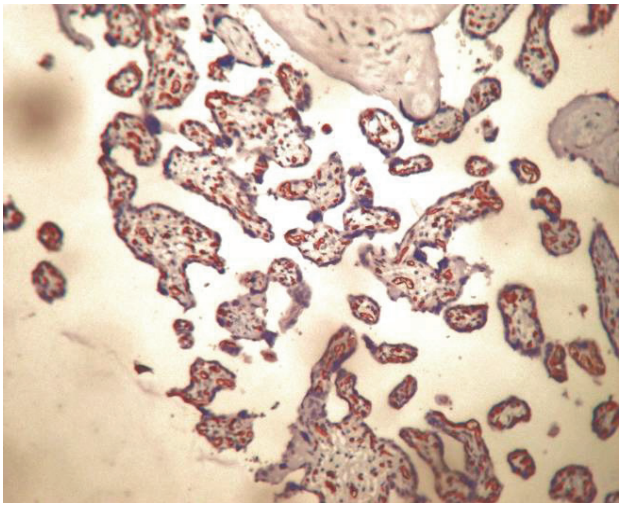

Figure 8 – Syncytial knots along the stem or distal villi with trophoblast cells invading the placental bed (IHC – CD34+, x 40).

We noticed the absence of any distal villous core and a lot of fibrous bridges who connected the intervillous spaces of one villus to another, giving a pseudolabyrinthine appearance to the villous tree – Figure 9.

In the lumina of the still preserved capillaries we recognised red blood corpuscles and the fibrous connective tissue proliferating and replacing the fetal blood sinusoids, in the terminal villi.The absence of the capillary wall structure, in the reduced number of villi exhibited some fetal nucleated red blood cells with the appearence to rise directly from the connective tissue stroma – Figure 10.

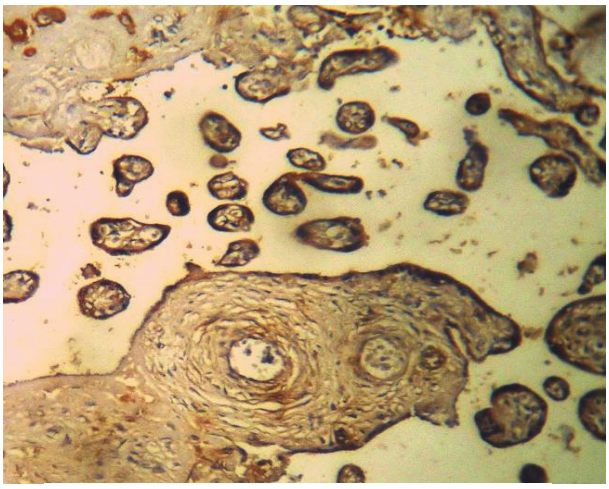

Figure 9 – Fibrin or bridging syncytial knots, pseudola- byrinthine appearance of the villous tree (IHC - Cytokeratin +, x 100).

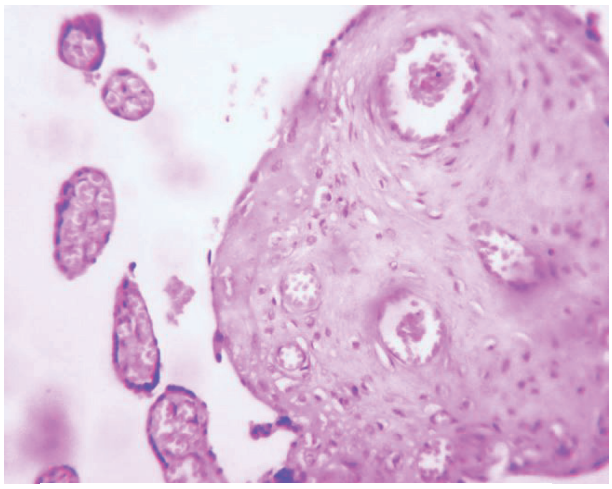

Figure 10 – Stromal vascular karyorrhexis with nuclear debris of fetal cells (H.E., x 200).

Discussions

PIH represents a real natural model of fetal malnutrition and hypoxia.The reduction of the vascular dimensions is constantly accompanied by significant structural disorders which have an impact upon the lumen of spiral arteriole[5] with changes in its tunica intima, media and fibrillary structures[6].These structural modifications are associated quasi-constantly with the PIH cases versus the normotensive cases, in which they are quite rare and isolated.

In our study the group N1 microscopical changes of the placentas showed diffuse hypoxia[7,8] histologically and modifications are suggestive for a predominantly hypoplastic mechanism. At a vascular level the first reaction to hypoxemia is the vasoconstriction. If the hypoxemia continues, it produces hypoplastic modifications, with immediate and late hemodynamic consequences[9]. The morphological modifications of the fetal vascular system may represent a main factor, for vascular affections of the future adult[10].

Conclusions

The morphological modifications of the feto-placental interface in the PIH represent a marker of important fetal and postnatal hemodynamic deficiencies. The hemodynamic status of the foetus and of the new-born baby by mothers suffering from PIH are characterized by hypoxia/ischemia with an immediate and late impact upon their cerebral development.

We consider that the above described lesions leed to many physiopathological consequences:

- important, and for long time, fetal blood stream reduction;
- a fetal oxygenation reduction with chronic hypoxemia, wich has a direct impact upon the cerebral development;
- fetal molecular signals by the mother with massive reaction from the pregnant woman body, leading to a bad evolution for both – fetus and mother.

A better understanding of the histological damages in preeclamtic feto-maternal interface, will change in the future, our medical assistance during the pregnancy.

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