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Immunohistochemical expresssion of caspase-3 in the syncytiotrophoblast of human preeclamptic placenta

Apoptosis (programmed cell death) is important in many aspects of reproduction and is considered to be fundamental for the normal pregnancy course and may be involved in the pathophysiology of pregnancy-related diseases (11). The balance between proliferation, maturation and programmed cell death is very important for normal placental growth, structure and function (7), thus for appropriate nutrition and oxygenation of the fetus and its normal development.

In preeclamptic pregnant women there is no adequate invasion of trophoblast and physiologic remodelling of spiral arteries, characteristic of normal pregnancy. Pathological adaptive changes in spiral arteries and disorders in the apoptotic process may result in increased sensitivity to vasoactive factors and decreased uteroplacental blood flow and may result in a variety diseases and pregnancy complications such as abortion, placental insufficiency, intrauterine fetal growth retardation or preeclampsia (1, 4, 5, 9).

The execution of apoptosis depends on the activation of caspases, a family of cysteine proteases. Caspase -3, also known as apopain, CPP32, is considered one of the central executor of molecules and effectors of apoptosis. Active caspase-3 has been found in human and mouse placentas (5).

The aim of this study was to examine immunoreactivity of the caspase-3 in the syncytiotrophoblast of the human placenta in patients with pregnancy complicated by preeclampsia.

The study was given the approval of the Board for Supervising Ethics in Medical Experiments at the Medical University of Lublin, Poland.

MATERIAL AND METHODS

The study was carried out on 10 patients with singleton pregnancies complicated by preeclampsia with appropriate-for-gestational-age weight infants (group P). Preeclampsia was determined by increased blood pressure >140 mm Hg systolic and >90 mm Hg diastolic in women who was normotensive before 20 weeks of gestation accompanied by proteinuria defined as the urinary excretion of more than 0.3 g protein in 24-hour specimen. The control group included 10 healthy normotensive patients with singleton uncomplicated pregnancies, without any renal, heart and vascular diseases and with normal laboratory tests (group K). All arterial blood pressure measurements in the control group were normal and did not exceed 135/85 mmHg. None of the patients from this group suffered from proteinuria. All patients in the study were non-smokers.

Immunohistochemistry was used to determine the localization of caspase-3 in the human placental tissue (antibody-Santa Cruz Biotechnology, CA, USA). Stained sections were evaluated according to the intensity of staining. Evaluation was performed blind, and values obtained are means of three independent assessments using a modification of the methods described by

Bergqvist et al. and Miturski et al. (2, 6). Semiquantitative immunohistochemical H-score (histologic staining score) values were calculated for each slide using the following equation: $\Sigma Pi = I \times (Pi/100)$.

H-score index was calculated from the staining intensity and frequency / number of positive cells per 100 scrutinised cells. The intensity of the staining (I) was evaluated in four groups: 0 – absent, 1 – weak, 2 – moderate, 3 – strong. The frequency (Pi) of positive cells per 100 scrutinised cells was classified into five different groups (I – 0%, II – 25%, III – 50%, IV – 75%, V – 100% of the positive cells). H-score index was used for the evaluation giving the maximal score of 3 points (ranged from 0 to 3).

All research was done *in vitro* after the delivery and it was not dangerous for delivering women and their infants. Data were expressed as mean +/- SD and were statistically analyzed with the computer program "Statistica 5.0". The level of statistical significance was established as $p<0.05$.

RESULTS

There were no statistically significant differences in patient profiles between groups in gravidity, parity, maternal age or in the birth weight of infants. Creatinine and urea levels were normal in all patients.

Systolic, diastolic and mean arterial blood pressure were statistically higher in the study group in comparison with the control group. The mean systolic blood pressure values were 154.00 +/– 8.90 mmHg in preeclamptic group and 107.50 +/– 7.16 mmHg in the control group. The mean diastolic blood pressure values were 103.00 +/– 4.60 mmHg in preeclamptic group, and 68.00 +/– 5.10 mmHg in the healthy controls. The mean arterial blood pressure values were 120.00 +/– 4.89 mmHg in preeclamptic patients and 81.17 +/– 4.54 mmHg in healthy patients. These differences were statistically significant ($p<0.001$).

Increased H-score index values for syncytiotrophoblast were found in preeclamptic patients, but these differences were not statistically significant ($p=0.15$). In preeclamptic patients the mean H-score was 0.757+/–0.521 versus 0.455+/–0.444 in healthy controls.

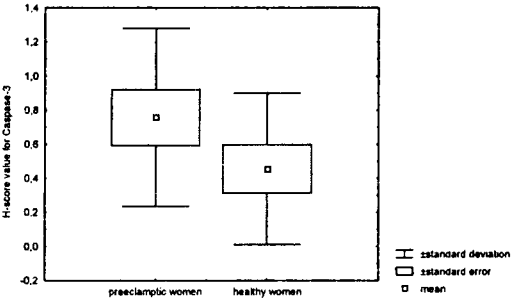

Fig. 1. H-score value for syncytiotrophoblast caspase-3 in the groups of the studied patients

DISCUSSION AND CONCLUSIONS

Apoptosis in human placenta and apoptotic mechanisms play a central role in placenta turnover (5, 8, 10). It is very important for normal placental development (3, 11), which is dependent upon the differentiation and invasion of the trophoblast. The caspases are the central executioners of apoptosis (11).

Huppertz et al. (3) reported that caspase 3, 6-8, and 10 are expressed in placenta. Caspase-3, considered as one of the central executier molecules, is responsible for cleaving various protein thereby disabiling important cellular structural, functional and repair processes (5). Then caspase-3 is activated and the apoptotic cells were executed (5).

We observed increased expression of caspase-3 in placental syncytiotrophoblast, but these differences were not statistically significant. Liu et al. (5) observed caspase-3 localization in syncytiotrophoblasts too. Also Wang et al. (12) found significantly higher caspase-3 activity in the patients with umbilical placental vascular disease compared with normal pregnancy. They suggest that caspase-3 signal transduction pathway seems to be involved in the mediation of endothelial apoptosis in HUVEC culture (12). Similar results were presented by Allaire et al. and Levy et al., who observed more apparent apoptosis in the syncytiotrophoblast and cytotrophoblast layer of villi from pregnancies complicated by preeclampsia than in trophoblast layer of villi from control pregnancies (1, 4).

According to our results and other reports it seems that enhanced expression of caspase-3 indicates the increased apoptosis in preeclamptic placentas, which may be involved in pathophysiology and sequele of preeclampsia.

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SUMMARY

The aim of this study was to examine immunoreactivity of the caspase-3 in the syncytiotrophoblast of the human placenta in preeclamptic pregnancy. The study was carried out on 10 patients with singleton pregnancies complicated by preeclampsia (group P). The control group included 10 healthy normotensive patients with singleton uncomplicated pregnancies, without any renal, heart and vascular diseases and with normal laboratory tests (group K). Immunohistochemistry was used to determine the localization and expression of caspase-3 in the human placental tissue. There were no statistically significant differences in patient profiles between groups in gravidity, parity, maternal age or in the birth weight of infants. Systolic, diastolic and mean arterial blood pressure were statistically higher in the study group in comparison with the control group. Increased H-score index values for caspase-3 in syncytiotrophoblast was found in preeclamptic patients, but these differences were not statistically significant. These results suggest that the apoptosis plays a role in the pregnancy complicated by preeclampsia.

Immunohistochemiczna ekspresja kaspazy-3 w syncytiotrofoblaście ludzkiego łożyska w ciąży powikłanej stanem przedrzucawkowym

Celem badań była ocena ekspresji kaspazy-3 w tkankach ludzkiego łożyska w ciąży powikłanej stanem przedrzucawkowym. Badaniami objęto 10 kobiet rodzących z ciążą powikłaną stanem przedrzucawkowym. Grupę kontrolną stanowiło 10 kobiet rodzących z fizjologicznym przebiegiem ciąży i prawidłowymi wartościami ciśnienia tętniczego krwi, bez schorzeń sercowo-naczyniowych i nerkowych oraz z prawidłowymi wynikami badań laboratoryjnych. Do oceny kaspazy-3 w tkankach łożyska zastosowano metodę immunohistochemii. Badane grupy kobiet nie różniły się pod względem płodności, rodności, wieku matki i urodzeniowej masy ciała noworodków. W grupie badanej odnotowano istotnie wyższe wartości ciśnienia tętniczego krwi w odniesieniu do grupy kontrolnej. W grupie kobiet z ciążą powikłaną stanem przedrzucawkowym odnotowano wyższy współczynnik H-score dla kaspazy-3, ale różnica ta nie była znamienna statystycznie. Uzyskane wyniki mogą sugerować znaczenie zjawiska apoptozy w ciąży powikłanej stanem przedrzucawkowym.