

The evaluation of selected indices of apoptosis in placentas from pregnancies complicated by fetal growth restriction

Zachowanie się wybranych parametrów oceny apoptozy w łożyskach pochodzących z ciąż powikłanych hipotrofią wewnątrzmaciczną.

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Abstract

Background: Fetal growth restriction is related to a high rate of prematurity and mortality. In cases of unknown origin utero-placental circulation changes are the main factor which is due to the changes in blood vessels. The understanding of the mechanism may help in further prevention of FGR.

Material and methods: The expression of bcl-2 and bax in normal pregnancies and complicated by FGR were compared. The study was conducted in 2005-2006 at The Medical University of Lodz – HRP Unit and The Kopernik Hospital – Lodz.

Bcl-2 and bax were estimated using an immunohistochemical method. Bcl-2 was estimated in trophoblast, bax in decidua and trophoblast.

Results: In a study group the mean value of bcl-2 in trophoblast was $37,04 \pm 10,51$, in a control group the mean value was $65,74 \pm 6,97$. The estimation of bax was done in trophoblast and decidua separately. In the group of FGR mean value of bax expression in trophoblast was $45,35 \pm 10,5$. In decidua the mean bax expression value was $24,11 \pm 7,3$. In controls in trophoblast the mean value was $12,53 \pm 7,54$, in decidua the mean expression of bax was $6,63 \pm 2,24$.

Conclusion: 1. Apoptosis in trophoblast is lower in normal pregnancy than in FGR.

2. Increased expression of pro-apoptotic proteins in placenta might be one of the reason for FGR development.

Key words: **apoptosis / bax / bcl-2 / fetal growth retardation – physiopathology / pregnancy complications – pathology / pregnancy complications – physiopathology / placenta – pathology / trophoblasts – pathology /**

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Otrzymano: 18.05.2007
Zaakceptowano do druku: 20.06.2007

Streszczenie

Ograniczenie wewnątrzmacicznego wzrastania płodu związana jest z wysokim odsetkiem wcześniactwa oraz powikłań okresu noworodkowego. W przypadkach o nieznannej etiologii najważniejszą rolę odgrywa ograniczenie przepływu krwi w krążeniu maczyno-płodowym związane ze zmianami w ścianach naczyń krwionośnych. Poznanie mechanizmu tworzenia się tych zmian może w przyszłości umożliwić skuteczne zapobieganie hypotrofii.

Materiał i metody: Badania zostały przeprowadzone w latach 2005-2006 w Klinice Patologii Ciąży Uniwersytetu Medycznego w Łodzi oraz WSS im. Kopernika w Łodzi.

Oceniono ekspresję bcl-2 oraz bax w łożyskach z ciąż fizjologicznych oraz z hypotrofii płodów.

Ekspresja bcl-2 oraz bax oceniana została z użyciem metod immunohistochemicznych.

Ekspresja bcl-2 ocenione zostało w trofoblaście a bax dla porównania w doczesnej i trofoblaście.

Wyniki: W grupie ciąż powikłanych hypotrofią płodów średnia wartość ekspresji bcl-2 w trofoblaście wynosiła $37,04 \pm 10,51$, w grupie porównawczej – ciąż fizjologicznych średnia wartość ekspresji wynosiła $65,74 \pm 6,97$.

W grupie ciąż powikłanych hypotrofią płodów średnia wartość ekspresji bax w trofoblaście wynosiła $45,35 \pm 10,5$. W doczesnej średnia ekspresja bax w tej grupie wynosiła $24,11 \pm 7,3$. W grupie porównawczej średnia ekspresja bax w trofoblaście wynosiła $12,53 \pm 7,54$, w doczesnej średnia ekspresja bax wynosiła $6,63 \pm 2,24$.

Wnioski: 1. Hamowanie procesu apoptozy w trofoblaście jest wyższe w ciąży fizjologicznej niż powikłanej hipotrofią wewnątrzmaciczną. 2. Wzrost ekspresji białek proapoptotycznych w łożysku może mieć wpływ na pojawienie się hipotrofii wewnątrzmacicznej płodu.

Słowa kluczowe: **apoptoza / bax / bcl-2 / opóźnienie rozwoju płodu – etiopatogeneza / ciąża – powikłania / łożysko – patofizjologia / łożysko – patomorfologia /**

Design

Fetal growth restriction (FGR) is connected not only to the decreased growth of newborns but also to the smaller weight and surface of placentas. Placental vessels are often restricted, their walls and epithelial cells are partly destroyed. These changes are the reason for the decreased exchange between maternal and fetal blood. This process lead to the increase of lipid peroxidation process and cell membranes destabilization [1, 2]. Imprinted genes might play a role in the more common medical conditions that affect the placenta, also in the placental response to maternal vascular underperfusion associated with IUGR [3].

Apoptosis (programmed cell death) is regulated by independent, often opposite processes which determine cell death [4]. A well known group of bcl proteins has two ways of action- pro- and anti-apoptotic. The proteins of bcl-2 group decrease apoptosis and protect mitochondrial membrane by decreasing cytochrome C release and blocking caspase 9 activity. Caspase 9 is one of initiating apoptosis enzymes from protease group- cysteine proteinase. Initiating and effectors caspases compose the death initiation signaling complex (DISC).

Pro-apoptotic action has another protein from bcl family - the bax protein. Bax as pro-apoptotic factor is activated by bid protein and decrease mitochondrial membrane permeability [5].

High activity of pro-apoptotic processes can restrict the sufficiency of utero-placental exchange and be the reason for the presence of fetal growth restriction.

Aim

The main aim of this study was to find the difference in apoptotic activity expressed by the activity of bcl-2 and bax in placenta from normal pregnancies and complicated by fetal growth restriction.

Materials and Methods

The study was conducted in 2005-2006 at The Medical University of Lodz – High Risk Pregnancy Unit and Obstetric Department of The Kopernik Hospital – Lodz.

The written approval of University Ethics Committee was obtained and the formal informed consent of all subjects was taken.

Two groups of patients were compared:

I study group – pregnant women in pregnancies complicated by fetal growth restriction determined by post natal diagnosis (weight of newborns in centile charts 9-5) – 21 placental fragments. We focused on cases of IUGR with unknown causes.

II group- controls – pregnant women with normal neonatal weight (weight of newborns in centile charts 25-75) – 11 fragments of placenta.

Excision 2x2cm, from central part of placenta, near to the umbilical cord insertion was taken in aseptic conditions, fixed in a formalin solution and absorbed in paraffin blocks.

Bcl-2 – anti-apoptotic protein, expression was estimated using an immunohistochemical method by monoclonal mouse antibodies anti human bcl-2 oncoprotein. To estimate bax expression polyclonal rabbit antibody anti human bax was used. Antibodies were produced by Dakocytomation.

Bcl-2 was estimated in trophoblast. The estimation of bax was done in two regions of placental fragments – decidua and trophoblast to compare these two regions.

A statistical analysis was done using non-parametric U-Mann-Whitney and t-Student' tests.

As statistically significant p value <0.05 was considered.

Results

The estimation of bcl-2 was done in trophoblast, by counting bright points in ten random zones of vision.

In the group of intrauterine growth restriction the quality

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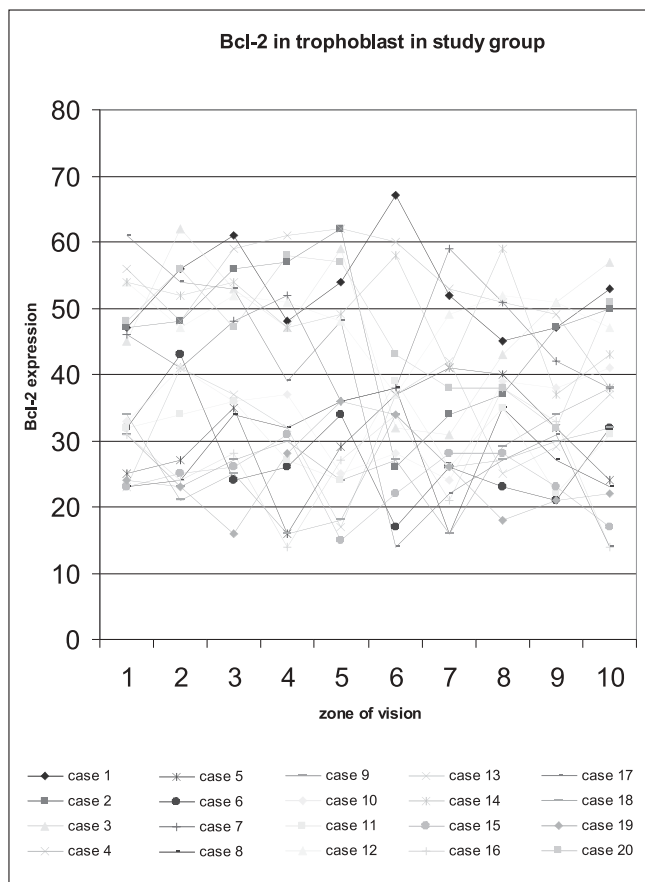


Figure 1. Bcl-2 expression in trophoblast in study group – values from 10 zones of vision.
Ekspresja bcl-2 w trofoblaście w grupie ciąży powikłanych hipotrofią wewnątrzmaciczną – wartości z 10 pól widzenia.

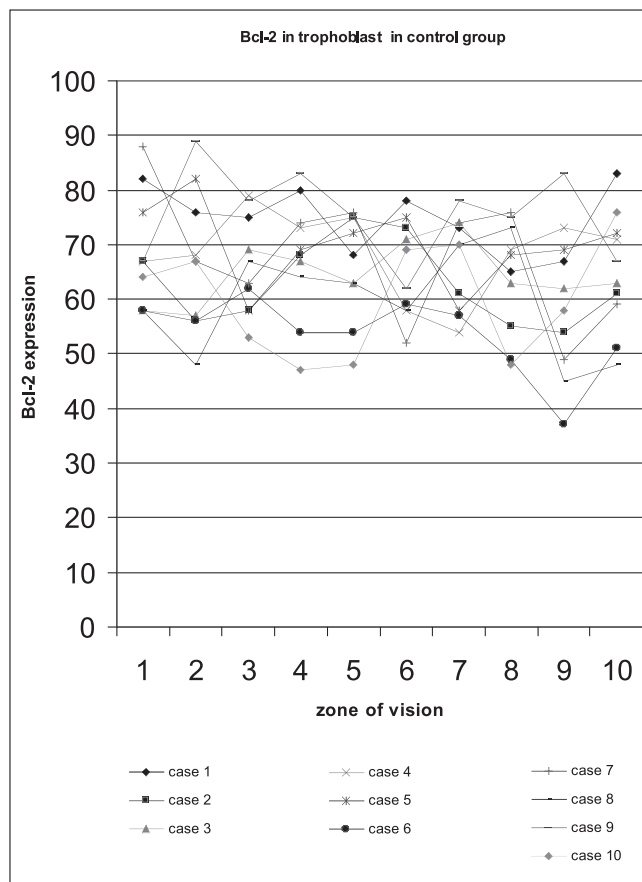


Figure 2. Bcl-2 expression in trophoblast in control group – values from 10 zones of vision.
Ekspresja bcl-2 w trofoblaście w grupie porównawczej – wartości z 10 pól widzenia.

of immunochemical reaction was incorrect in one case and the evaluation was done in 20 fragments.

The lowest mean expression of bcl-2 was 23,8, the highest 53,7 and the mean value was $37,04 \pm 10,51$ (Figure 1).

In the control group the estimation of bcl-2 value was done successfully in 10 fragments of placenta also in ten random zones of vision. The lowest mean expression of bcl-2 was 37,2, the highest 89,3 and the mean value was $65,74 \pm 6,97$ (Figure 2).

In a group of healthy women and normal-weight newborns- the expression of bcl-2 was higher than in FGR group. The difference between bcl-2 expression in compared groups was statistically significant ($p < 0.05$) (Figure 3).

The estimation of bax was done in trophoblast and decidua separately, by counting bright points in ten for trophoblast and six for decidua random zones of vision. In the group of intrauterine growth restriction the quality of immunochemical reaction was incorrect in one case and the evaluation was done in 18 fragments. The lowest mean expression of bax in study group in trophoblast was 28,2, the highest 70,0 and the mean value was $45,35 \pm 10,5$ (Figure 4).

In the control group in trophoblast the estimation of bax value was done successfully in 8 fragments of the placenta. The lowest mean expression of bax was 8,0, the highest 16,3, the mean value was $12,53 \pm 7,54$ (Figure 5).

In a group of healthy women normal-weight newborns the expression of bax was lower than in FGR group. The difference between bax expression in trophoblast in compared groups was statistically significant ($p < 0.05$) (Figure 6).

In decidua part of placenta in the study group the lowest mean expression of bax was 20,0, the highest 45,3 and the mean value was $24,11 \pm 7,3$ (Figure 7).

In decidua part of placenta in the control group the lowest mean expression of bax was 2,3, the highest 24,0 and the mean value was $6,63 \pm 2,24$ (Figure 8).

In the group of healthy women normal-weight newborns the expression of bax was lower in, than in FGR group. The difference between bax expression in compared groups was statistically significant ($p < 0.05$) (Figure 9).

In both areas – trophoblast and deciduas the expression of bax was significantly higher in placentas from FGR – complicated pregnancies.

Discussion

In FGR complicated by preeclampsia the expression of matrix-metaloproteinases was reduced, especially close to the spiral arteries [6]. This suggests that high expression of bax in FGR placentas in our study could be one of the reasons for this complication of pregnancy.

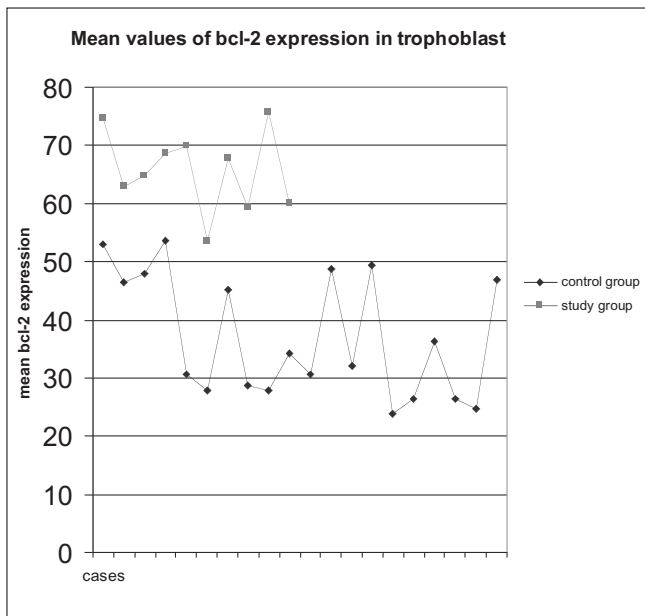


Figure 3. Mean values of trophoblastic bcl-2 expression in compared groups. (t-Student test)
Średnie wartości ekspresji bcl-2 w porównywanych grupach.

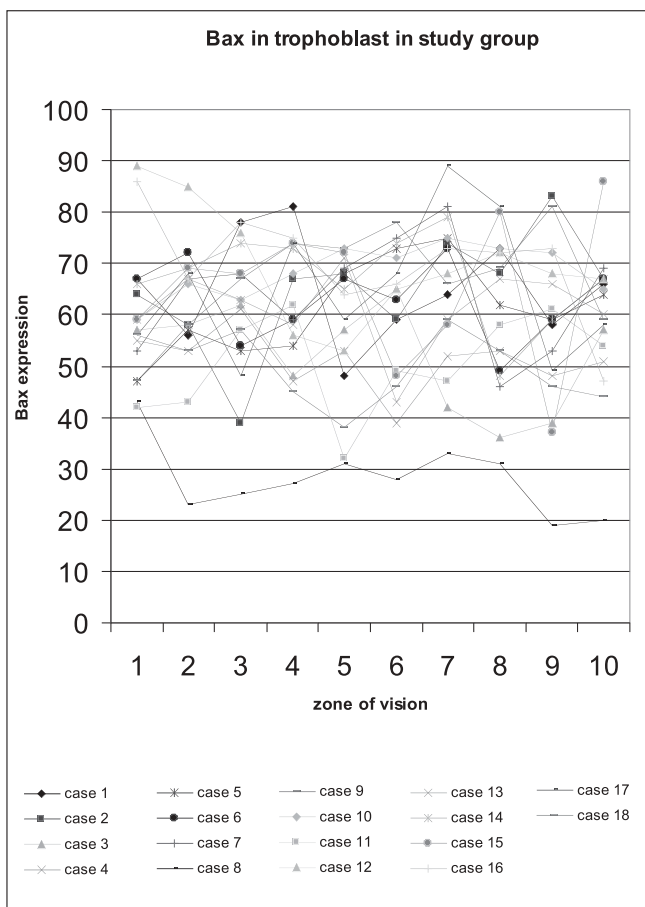


Figure 4. Bax expression in trophoblast in study group – values from 10 zones of vision.
Ekspresja bax w trofoblaście w grupie ciąży powikłanych hipotrofią wewnątrzmaciczną – wartości z 10 pól widzenia.

Apoptosis has been described in trophoblast and the importance of apoptosis cascade for the normal function of the trophoblast has become obvious. One feature of serious conditions such as intrauterine growth restriction is a change in apoptosis regulation in extravillous trophoblast resulting in the shedding into the maternal circulation [6]. High expression of bax and low of bcl-2 in extravillous trophoblast in our study stays in agreement with Huppertz study [5]. Trophoblast invasion into the placental bed in early-onset intrauterine growth restriction is limited by increased apoptosis, resulting in narrower spiral arteries [8].

Madazli found that in placental bed (maternal decidual tissue) apoptosis assessed by the TUNEL method was higher if compared with control placentas [9], which remains in agreement with our results. Stepan study shows that the proapoptotic proteins BNip3 and Nix are expressed in human placenta with placental dysfunction and FGR. It has been shown that placental hypoxia as well as apoptosis is a pathogenetic factor for intrauterine growth retardation [10].

Immunohistochemical analysis performed to examine the expression of proteins related to apoptosis, including the active form of bax shows in Endo data [11] no significant difference in bax expression in normal and FGR placentas.

In our study the expression of bax in a group of healthy women and normal-weight newborns was lower in both regions – trophoblast and deciduas, than in FGR group.

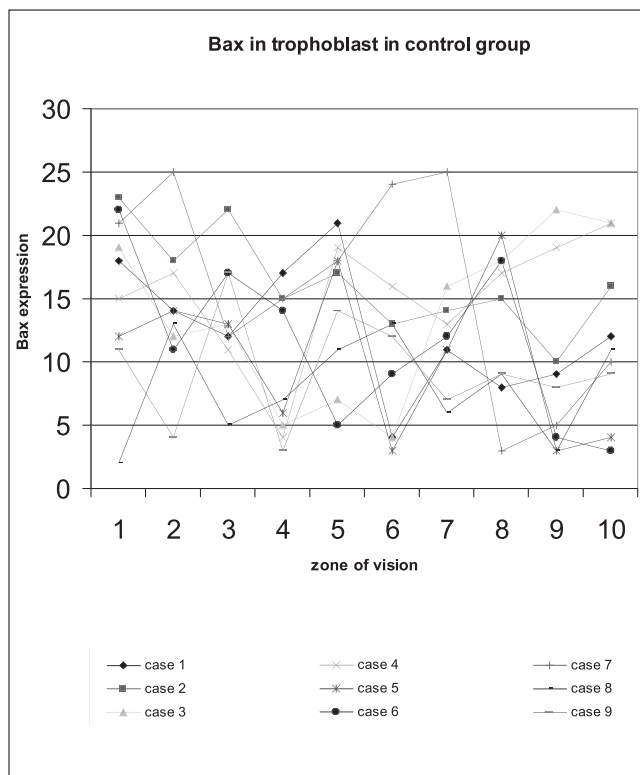


Figure 5. Bax expression in trophoblast in control group – values from 10 zones of vision.
Ekspresja bax w trofoblaście w grupie kontrolnej – wartości z 10 pól widzenia.

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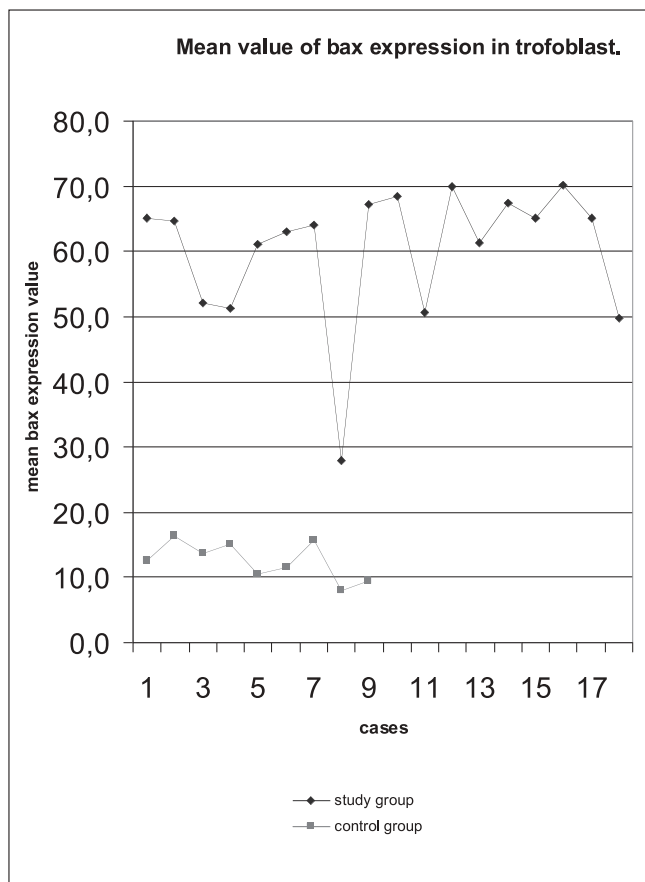


Figure 6. Mean values of trophoblastic bax expression in compared groups. (t-Student test)
Średnie wartości ekspresji bax w trofoblaście w porównywanych grupach.

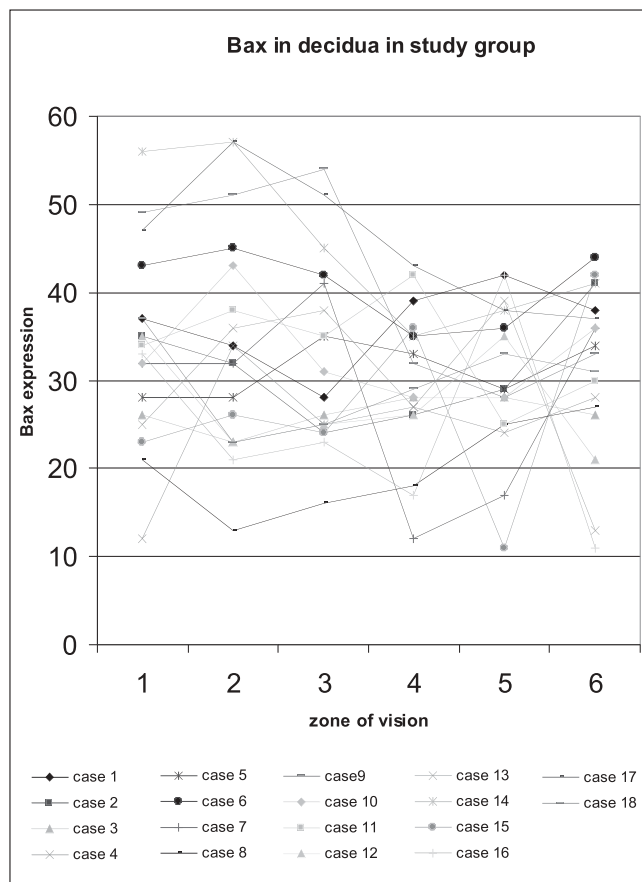


Figure 7. Bax expression in decidua in study group – values from 6 zones of vision.
Ekspresja bax w doczesnej w grupie ciąży powikłanych hipotrofią wewnątrzmaciczną – wartości z 6 pól widzenia.

The trophoblast cells need to be susceptible to undergo apoptosis which decides of endovascular trophoblast apoptosis and seems to be highly relevant for a correct transformation of spiral arteries [12].

The analysis of apoptotic cells by immunohistochemistry in fetal membranes showed a significant increase in FGR-affected fetal membranes compared to normal [13]. The density of apical microvilli appears considerably reduced in growth retardation cases, the underlying basal membrane appears significantly thicker than that of normal syncytiotrophoblast and apoptosis is a possible cell deletion mechanism in growth restriction [14]. In our study both trophoblast and decidua the expression of estimated pro-apoptotic proteins was statistically higher in FGR group which is in agreement with data of other authors [11, 12, 13, 15].

Apoptosis was also increased in the placentas of smoking mothers with growth-restricted infants and the reduction in blood flow had previously been shown to increase apoptosis, and it is possible that this could be one of the mechanisms playing a role in the growth restriction. In our study all pregnant women were non-smokers but placental changes in apoptosis were similar [15].

Conclusions

1. Apoptosis in trophoblast was lower in normal pregnancy than in FGR.
2. Increased expression of pro-apoptotic proteins in FGR shows an increase of apoptosis in the placenta.

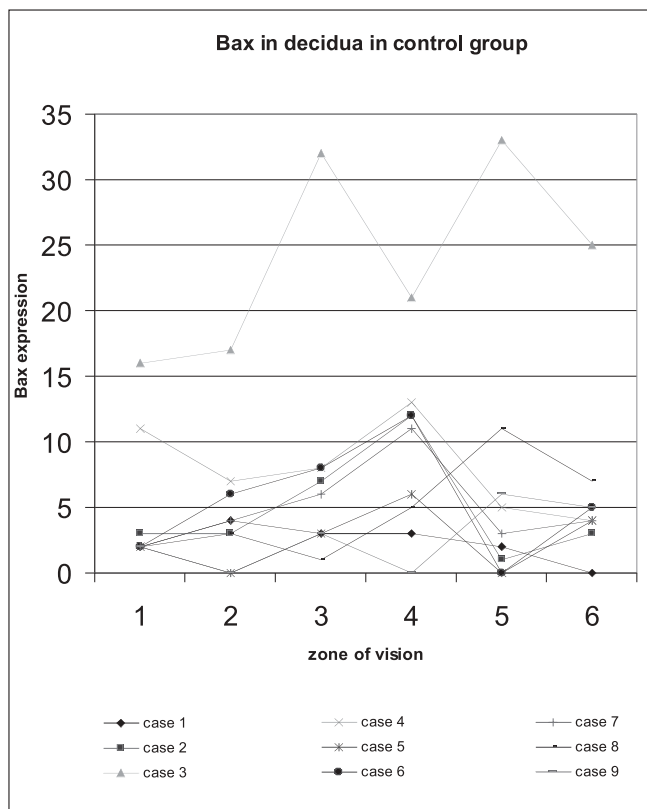


Figure 8. Bax expression in decidua in control group – values from 6 zones of vision.
Ekspresja bax w doczesnej w grupie kontrolnej – wartości z 6 pól widzenia.

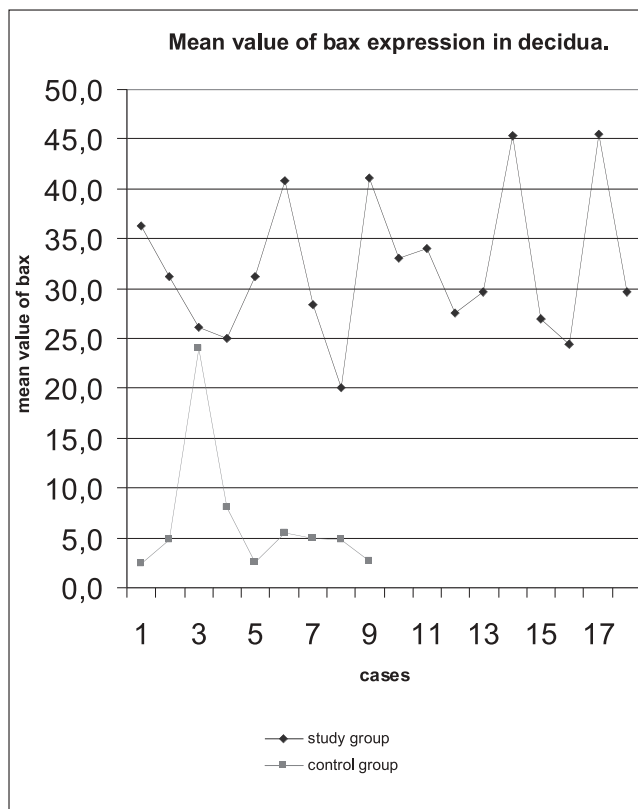


Figure 9. Mean values of decidual bax expression in compared groups.
(t-Student test)
Średnie wartości ekspresji bax w porównywanych grupach.

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Praca została sfinansowana z funduszy prac własnych U.M. nr: 502-11-364.