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AKADÉMIAI KIADÓ

The role of apoptosis in the complex pathogenesis of the most common obstetrics and gynaecology diseases

P. KOVÁCS[1,2], JÓZSEF GÁBOR JOÓ[2,3*], V. TAMÁS[2], Z. MOLNÁR[2], D. BURIK-HAJAS[2], J. BÓDIS[2,4] and L. KORNYA[2,5]

[1] Clinical Research Units Hungary, Miskolc, Hungary

[2] Doctoral School of Health Sciences, Faculty of Health Sciences, University of Pécs, Pécs, Hungary

[3] First Department of Obstetrics and Gynaecology, Semmelweis University, Budapest, Hungary

[4] Hungarian Academy of Sciences, University of Pécs (MTA-PTE), Human Reproduction Scientific Research Group, University of Pécs, Pécs, Hungary

[5] Central Hospital of Southern Pest, National Institute of Hematology and Infectious Diseases, Budapest, Hungary

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ABSTRACT

Purpose: We aimed to assess the etiological role of apoptotic genes Bcl-2 and Bax in the background of major obstetric and gynaecological diseases. *Methods:* Placental tissue samples were collected from 101 pregnancies with intrauterine growth restriction and 104 pregnancies with premature birth with 140 controll samples from term, eutrophic newborns. In addition, gene expression assessment of the genes Bax and Bcl-2 was performed in 101 uterine leiomyoma tissue samples at our disposal with 110 control cases. Gene expression levels were assessed by PCR method. *Results:* The expression of the Bcl-2 gene was decreased in placental samples with intrauterine growth restriction. Significant overexpression of the proapoptotic Bax gene was detected in samples from premature infants. Antiapoptotic Bcl-2 gene expression was found to be significantly increased in fibroid tissues. *Conclusion:* Apoptosis plays a crucial role in the development of the most common OB/GYN conditions. Decrease in the placental expression of the antiapoptotic gene Bcl-2 may upset the balance of programmed cell death.

* Corresponding author. Clinical Research Units, H-2132 Thököly str. 15, Göd, Hungary. Fax: +36 46798264, E-mail: joogabor@hotmail.com

KEYWORDS

gene expression, placenta, apoptosis, intrauterine growth restriction, premature birth, uterine leiomyoma, Bcl-2, Bax

ABBREVIATIONS

IUGR, intrauterine growth restriction, Bcl-2, B cell lymphoma 2, BH, Bcl-2 homologue, BMI, body mass index

INTRODUCTION

Programmed cell death (apoptosis) plays an important role during the development of the placenta and in the regulation of its ageing during pregnancy [35]. The regulation of programmed cell death is complex; both proapoptotic (stimulating programmed cell death) and antiapoptotic (inhibiting programmed cell death) genes are involved. Members of the Bcl-2 (B cell lymphoma 2) protein family play a prominent role in this complex regulatory system [8, 21, 25, 39]. Based on structure and function, genes of the Bcl-2 family may also be classified as genes with pro- and anti-apoptotic effect [4, 16, 37]. Genes with antiapoptotic effect include Bcl-2, Bcl-xL (Bcl-extra long), A1, Bcl-w and Boo, whereas the proapoptotic group includes Bax, Bak, and Box genes. Bcl-2 from the antiapoptotic group, and Bax from the proapoptotic group exert the strongest biological effect; accordingly, we simply traced back the regulation and balance of the apoptotic process in the investigated conditions to the establishment of the balance between these two genes, and to the change in their activity [9, 38]. Several studies have confirmed that the role of apoptosis increases during pregnancy with increasing gestational age, and that this is related to the change in activity of the members of the Bcl-2 gene family [19, 43, 45]. Development and ageing of the placenta are both essential for normal gestation and near-term delivery. Several gestational pathological states have been related to the modified apoptotic activity of trophoblast cells, such as intrauterine growth restriction, premature birth or preeclampsia [1, 11, 14, 22, 23].

Changes in apoptotic activity play a role in the development of both benign and malignant tumours. Uterine leiomyomas (benign tumours of the womb originating from smooth muscle) are the most frequent benign tumours in women. Tumour growth is determined by increased cell proliferation and/or decreased intensity of apoptosis. Based on previous data in the literature, it has been suggested that Bcl-2 and Bax gene expression is altered in fibroids compared to normal myometrium [12, 27, 30, 47], although this has not yet been confirmed on a larger patient population.

Intrauterine growth restriction (IUGR) is present when foetal weight falls below the 10th percentile of the standard for sex and gestational age [17, 20, 41, 46]. IUGR may result from placental dysfunction, foetal malformations (e.g. chromosomal abnormalities), intrauterine infection (e.g. cytomegalovirus infection, toxoplasmosis), maternal diseases (e.g. kidney diseases, autoimmune diseases) or environmental factors.

Premature birth is present when the pregnancy is terminated by birth before the 37th gestational week. When gestational age cannot be determined accurately, premature birth is defined if the infant's birth weight stays below 2,500 g [13, 31, 42, 44].

Uterine fibroid is a benign tumour originating from the smooth muscle cells of the womb, which develops mostly in women of child-bearing age, and its incidence increases with

age [6, 26, 28, 34]. The most common symptoms caused by uterine fibroid are irregular bleeding and pelvic pain. It may also lead to obstetric complications like infertility, recurrent abortions and premature delivery, as well as to the compression of other pelvic organs. Although no gene responsible for the development of uterine fibroids not being part of a syndrome has yet been identified, familial accumulation reported in several studies indicates genetic determination of the condition [18].

The aim of our study was to compare Bax (proapoptotic) and Bcl-2 (antiapoptotic) gene expression patterns of placental samples from pregnancies with intrauterine growth restriction or premature birth with those from healthy, eutrophic and mature pregnancies. It was also our aim to assess the expression patterns of the most relevant Bax and Bcl-2 genes depending on the severity of intrauterine growth restriction, and review clinical data that may significantly contribute to the interpretation of gene expression results as well as possible interactions of suspected etiological factors in the background of premature birth. Additionally, our aim was to assess Bcl-2 and Bax gene activity in uterine fibroid tissue in a large number of cases and to analyse it in view of clinical and demographical data.

MATERIALS AND METHODS

Patients (IUGR)

During our 1-year study, gene expression results of 101 placenta samples obtained during the birth of infants with intrauterine growth restriction treated at the Semmelweis University 2nd Department of Obstetrics and Gynaecology were compared to the gene expression levels of placental samples obtained during the birth of 140 eutrophic newborns. In addition, basic clinical and demographical data were also analysed. According to one of the study aspects, pregnancies with growth restriction were classified into two groups by severity, depending on the birth weight ranging between 0–5th and 5–10th percentile. After excluding intrauterine infections, chromosome disorders, other foetal developmental disorders, maternal malnutrition, multiple pregnancies and placental disorders, functional placental disorder was suggested as the cause of intrauterine growth restriction.

Patients (premature birth)

During the one-year study, gene expression was investigated in 104 placental samples obtained from pregnancies with premature birth. The diagnostic criteria for premature birth were gestational age younger than 37th gestational week and/or birth weight below 2,500 g. Cases of intrauterine growth restriction, multiple pregnancy, developmental disorders, maternal anatomical disorder or induced premature birth were excluded from the study. In the investigated cases, premature birth commenced by premature uterine activity and/or premature rupture of the membranes.

Placental sampling, clinical and demographic data collection

In each case, a tissue sample, approximately 2 × 2 × 2 cm (8 cc) in size, was obtained from the placenta at a distance of approximately 3 cm from the origin of the umbilical cord. Samples were stored at −70 °C until further gene expression assessment. Assessed clinical data: maternal age,

paternal age, obstetrics history, genetic history, other diseases, mother's birth weight, gestational age at birth, foetal sex, weight gain during pregnancy, change of body mass index (BMI) during pregnancy, carbohydrate metabolism disorder during pregnancy, other obstetrical disease during pregnancy, infant's weight, Apgar score, smoking, and group B Streptococcus screening. Regarding the way of delivery no distinction was made either in placental sampling or in gene expression analysis.

Analyses were performed with the knowledge and consent of expectant mothers in all cases, which was confirmed by their signature (Ethical approval number: TUKEB 114/2009).

Patients (uterine leiomyomas)

During the one-year study, gene expression results of tissue samples obtained from 101 women undergoing surgery for uterine fibroid at the Semmelweis University 1st Department of Obstetrics and Gynaecology were compared to the gene expression values of tissue samples obtained from 110 women undergoing hysterectomy for causes other than uterine fibroid. The preoperative diagnosis of uterine leiomyoma was established by gynaecological examination and sonography in all cases. The type of surgery was determined according to clinical aspects, patient age, possible further family planning and location, size and number of fibroid(s). The results of gene expression assessment were not sorted according to the type of surgery (transvaginal hysterectomy, transabdominal hysterectomy, fibroid enucleation). During the interpretation of gene expression results only the cases where the diagnosis of uterine fibroid was also confirmed by postoperative histological evaluation were included. Only those cases were assigned as controls where the indication for hysterectomy was other than uterine fibroid or malignant tumour. The fulfilment of these criteria was confirmed by histological evaluation in all cases.

Leiomyoma tissue sampling and clinical and demographic data collection

In the cases of myoma enucleation, tissue samples $1 \times 1 \times 1$ cm (1 cc) in size were obtained from the removed tumour when possible. Samples were stored at -70 °C until further gene expression assessment. When multiple fibroids were removed, samples were obtained from each of the resected tumours. In these cases, the average of the gene expression values of the individual fibroids was calculated and considered as one value. For hysterectomy, the tissue sample was obtained from the fibroid when possible; or if not, sampling was performed in the region of the fundus of the removed womb at a volume of 6–8 cc. For control cases, a tissue sample $2 \times 2 \times 2$ cm in size was removed from the region of the fundus of the uterus.

Assessed clinical data: age, family history of uterine fibroids, time of the first period, number of pregnancies, number and way of deliveries, (total) length of lactation period(s) after pregnancy (pregnancies), unsuccessful pregnancies (spontaneous abortion, missed abortion, artificial abortion), oral contraception, preoperative sonographic result, number, size and location of uterine fibroids, type of the surgery, and the results of histological evaluation. For pregnancies, the total amount of time spent being pregnant was used, given in weeks. An average of 37 gestational weeks per pregnancy was used for our analysis. (The time of pregnancies ending in termination by spontaneous or artificial abortion, in view of their shortness, was not taken into consideration).

Gene expression assessment (IUGR, premature birth and uterine fibroid)

The total amount of RNA present in the placental and fibroid samples was extracted with the Quick RNA Micropep Kit (Zymo Research), then RNA concentration was measured by the NanoDrop spectrophotometer (NanoDrop). Reverse transcription (RT) was performed in a final volume of 20 μL: using 5 μg of total RNA, 75 pmol of random hexamer primer, 10 mM of dNTP (Invitrogen), 20 U M-MuLV Reverse Transciptase enzyme (MBI Fermentas) and 1× buffer (MBI Fermentas). The reaction mixture was incubated at 42 °C for 2 h, then the enzyme was inactivated at 70 °C for 15 min.

The reverse transcription reaction mixture was diluted threefold with nuclease-free water. For real-time PCR, 1 μL of diluted cDNA (the equivalent of ~15 ng RNA) and 1 × SYBR Green Master Mix (Applied Biosystems) were used. Primers were designed using the Primer Express Software (Applied Biosystems) (primer sequences are presented in Table 1). Real-time PCR reaction was performed with 1 μL cDNA, 1 pmol, gene-specific Forward and Reverse primers and 1 × SYBR Green PCR Master mix in a final volume of 20 μL. All real-time PCR reactions were carried out with MX3000 Real-time PCR (Stratagen) equipment according to the following protocol: 40 cycles, denaturation at 95 °C for 15 s, primer-annealing, chain lengthening and detecting at 60 °C for 60 s. The relative expression of each gene was normalised for the human *β-actin* gene.

Statistical analysis

The Stratagen MX3000 real time PCR software (Stratagen) was used to calculate gene expression values. Threshold cycle value (*Ct*) is defined as the reaction time (real-time PCR cycle time) by which the evaluating software detects a fluorescent signal that unequivocally deviates from the baseline signal. The delta *Ct* value (ΔCt) demonstrates the difference between the *Ct* value of the target gene measured in the assessed sample and that of the internal control gene ($\Delta Ct = Ct_{\text{assessed gene}} - Ct_{\text{internal control gene}}$). The α-value characterises the relative difference of target genes between two different samples ($\Delta Ct_{\text{sample 1}} - \Delta Ct_{\text{sample 2}}$). The natural logarithm of the $2^{\dot{\alpha}}$ value shows how the relative amount of the target gene RNA compares between two samples. The UCL (upper confidence limit) and the LCL (lower confidence limit) refer to the highest and lowest relative differences between assessed samples, respectively.

A two-sample *t*-test was used to assess gene expression results (confidence interval 95%). The degree of freedom was determined by Welch–Satterthwaite approximation. Gene expression

Table 1. Primers and sequences used for real-time PCR reactions in the gene expression assessments for IUGR, premature birth and leiomyoma uterine

Name and ID of gene	Forward primer	Reverse primer
Bcl-2 (NM_000633)	5′-ATGTGTGTGGAGAGCGTCAACC-3′	5′-TGAGCAGAGTCTTCAGAGACAGCC-3′
Bax (NM_004324)	5′-CCTTTTCTACTTTGCCAGCAAAC-3′	5′-GAGGCCGTCCCAACCAC-3′
β-Actin (M10277)	5′-GGCACCCAGCACAATGAAG-3′	5′-GCCGATCCACACGGAGTACT-3′

values were classified into the following groups: (1) overactive gene: if the Ln value of the calculated data >1, $P < 0.05$; (2) underactivity: if the Ln value of the calculated data <−1, $P < 0.05$; (3) unchanged activity: if the Ln value of the calculated data <1, >−1, $P < 0.05$. GraphPad Prism 3.0 (GraphPad Software Inc, La Jolla, CA, USA) software was used for all statistical evaluations.

To analyse demographic and clinical data, we created models with mathematical statistical tools, using the SPSS software package. As multidimensional process we applied logistic regression (due to the dichotomous dependent variables), analysis of variance (ANOVA) and linear regression. Correlations were considered significant when $P < 0.05$.

RESULTS

Clinical data (IUGR)

Male:female ratio was 0.58 (64 boys; 37 girls) and 1.09 (boys: 73; girls: 67) in the intrauterine growth restriction group and in the eutrophic control group, respectively ($P < 0.05$). The median age of expectant mothers did not differ significantly (30.82 ± 4.34 years/IUGR/vs 31.45 ± 3.12 years/eutrophic/; $P > 0.05$), whereas significant differences were observed regarding weight gain during pregnancy (14.8 kg/eutrophic/vs 10.9 kg/IUGR/) and change in body mass index (+5.3/eutrophic/vs 4.1/IUGR/) of pregnant women ($P < 0.05$).

Regarding the birth weight of expectant mothers, our data confirmed that the birth weight (median: 2,830 g) of women delivering infants with birth weight between 0–5th percentile was significantly lower than those delivering infants with birth weight between 5 and 10th percentile (median: 3,120 g) ($P < 0.05$).

Clinical data (premature birth)

Boy:girl ratio was 0.89 (49 boys; 55 girls) and 1.09 (73 boys; 67 girls) among premature infants and in the control group, respectively ($P > 0.05$). Median age of women giving birth prematurely did not show any significant difference compared to the control group (30.7 ± 5.20 years/premature birth/vs 31.4 ± 3.12 years/mature birth/) ($P > 0.05$).

Prenatal weight gains of pregnant women delivering mature and premature infants, that were compared during the study, showed a significant difference in accordance with the shorter gestational period preceding premature births (11.6 ± 4.6 kg/premature birth/vs 14.7 ± 2.6 kg/mature birth/) ($P < 0.05$).

The rate of regular smokers with pregnancies terminating in premature birth was 26.9% (28/104) compared to 7.1% in the mature control group (10/140) ($P < 0.05$).

Premature birth commenced with premature rupture of the membranes in 70.2% of cases (73/104) and spontaneous uterine activity in 29.8% of cases (31/104).

Clinical data (uterine leiomyoma)

Patients treated for uterine leiomyoma were significantly younger (median age) than patients in the control group (47.5 ± 12.1 years vs 54.7 ± 10.2 years; $P < 0.05$).

Regarding the median length of total time spent pregnant in weeks, our data showed that women with leiomyomas spent a significantly shorter time in pregnancy (105.1 ± 8.2 weeks) than patients in the control group (127.2 ± 9.1 week) ($P < 0.05$).

Table 2. The incidence of the most common symptoms of leiomyoma uterine in the myoma and control group

		Myoma cases		Control cases	
		n	%	*n*	%
Symptoms	Abdominal pain: negative; bleeding disorder: negative	26	25.7	56	51.1
	Abdominal pain: negative; bleeding disorder: positive	28	27.0	21	19.2
	Abdominal pain: positive; bleeding disorder: negative	12	12.2	16	14.4
	Abdominal pain: positive; bleeding disorder: positive	35	35.1	17	15.3
	Total	**101**	100	**110**	100

Nulliparity was significantly more frequent among women with fibroids (32/101; 31.7%) than in the control group (6/110; 5.5%) ($P < 0.05$).

Lactation period following pregnancy was, understandably, significantly shorter than in the control group (2.4 ± 1.2 months vs 5.1 ± 2.2 months; $P < 0.05$).

The prevalence of the two most common symptoms of uterine leiomyomas (bleeding disorder, lower abdominal pain) together, as well as that of the bleeding disorder on its own was significantly higher ($P < 0.05$) in the group of patients with uterine fibroids than among control cases (Table 2).

Of the 101 leiomyoma cases, 40 enucleations (39.6%) and 61 hysterectomies (60.4%) were performed.

Gene expression assessment

The expression of the proapoptotic Bax gene did not show any significant differences in the samples from pregnancies with IUGR, whereas the antiapoptotic Bcl-2 gene showed a significant

Table 3. Bax- and Bcl-2 gene expression activities in the placenta samples with intrauterine growth restriction compared to control samples from eutrophic pregnancies

Name of gene	$\Delta Ct_{\text{eutrophic}} \pm \text{SE}^{(\text{I.})}$	$\Delta Ct_{\text{IUGR}} \pm \text{SE}^{(\text{II.})}$	α value ± $\text{SE}(\alpha)^{(\text{III.})}$	Ln 2^{α}	LCL	UCL	*P*	Gene expression changes
Bax	3.18 ± 0.82	4.04 ± 0.67	−0.86 ± 0.5	−0.59	0.43	−1.21	0.06	Not changed in function
Bcl-2	3.18 ± 0.82	6.32 ± 0.86	−3.14 ± 0.81	−2.17	0.85	−3.79	0.04	Underactivity

UCL: upper confidence limit.
LCL: lower confidence limit.
Significant difference: $P < 0.05$.
(I.) $\Delta Ct_{\text{eutrophic}} = Ct_{\text{assesed gene}} - Ct_{\beta\text{-actin}}\ \text{n}_{\text{IUGR}} = 101$.
(II.) $\Delta Ct_{\text{IUGR}} = Ct_{\text{assesed gene}} - Ct_{\beta\text{-actin}}\ \text{n}_{\text{eutrophic}} = 140$.
(III.) $\alpha = \Delta Ct_{\text{eutrophic}} - \Delta Ct_{\text{IUGR}}$.

Table 4. Bax and Bcl-2 gene expression of placental samples from foetuses with severe intrauterine growth restriction classified into the 0–5th percentile weight range, compared to similar parameters of foetuses with milder intrauterine growth restriction of 5–10th percentile

Name of gene	$\Delta Ct_A \pm SE$	$\Delta Ct_B \pm SE$	α value $\pm$ SE(α)	Ln 2^{α}	P	Gene expression changes
Bax	4.32 ± 0.46	3.76 ± 0.3	0.56 ± 0.28	0.38	0.06	Not changed in function
Bcl-2	6.92 ± 0.58	5.7 ± 0.64	1.22 ± 0.53	0.84	0.07	Not changed in function

A: 5–10 percentile IUGR placental sample; $n_{total} = 101$ ($n_A = 60$, $n_B = 41$).
B: 0–5 percentile IUGR placental sample.
$\Delta Ct_A = Ct_{assesed\ gene} - Ct_{\beta\text{-actin}}$;
$\Delta Ct_B = Ct_{assesed\ gene} - Ct_{\beta\text{-actin}}$;
$\alpha = \Delta Ct_A - \Delta Ct_B$;
Significant difference: $P < 0.05$.

underactivity in the placenta samples with IUGR compared to control samples from eutrophic pregnancies ($P < 0.05$) (Table 3).

No significant differences could be shown in Bax and Bcl-2 gene expression of placental samples from foetuses with severe IUGR classified into the 0–5th percentile weight range, compared to similar parameters of foetuses with milder intrauterine growth restriction of 5–10th percentile (Table 4).

Regarding gene expression activity of placenta samples from premature infants, a significant overexpression of the proapoptotic Bax gene could be detected compared to gene expression activity in the mature control group ($P < 0.05$), whereas the antiapoptotic Bcl-2 gene did not show any significant change in activity (Table 5).

Depending on gestational age, placental Bax and Bcl-2 gene expression values compared to the placental values from term births were detected as follows: no significant difference was present in the activity of antiapoptotic Bcl-2 gene in premature births at weeks 24–28, 28–32 and

Table 5. Bax and Bcl-2 gene expression activities of placenta samples from premature infants compared to gene expression activity in the mature control group

Name of gene	$\Delta Ct_{eutrophic} \pm SE^{(I.)}$	$\Delta Ct_{premature} \pm SE^{(II.)}$	α value $\pm$ SE(α)$^{(III.)}$	Ln 2^{α}	LCL	UCL	P	Gene expression changes
Bcl-2	2.76 ± 0.48	2.53 ± 0.7	−0.23 ± 0.6	−0.15	−1.12	0.91	0.07	Not changed in function
Bax	2.97 ± 0.8	1.02 ± 0.65	1.95 ± 0.72	1.35	0.82	2.13	0.04	Overactivity

UCL: upper confidence limit.
LCL: lower confidence limit.
Significant difference: $P < 0.05$.
(I.) $\Delta Ct_{eutrophic} = Ct_{assesed\ gene} - Ct_{\beta\text{-actin}}$; $n_{premature} = 104$; $n_{eutrophic} = 140$.
(II.) $\Delta Ct_{premature} = Ct_{assesed\ gene} - Ct_{\beta\text{-actin}}$;
(III.) $\alpha = \Delta Ct_{eutrophic} - \Delta Ct_{premature}$.

Table 6. Bax and Bcl-2 gene expression activities of placenta samples from premature infants compared to gene expression activity in the mature control group based on gestational age

n	Gestational age	Bax Ln 2^{α}	*P*	Bcl-2 Ln 2^{α}	*P*
15	Week 24–28.	0.87	0.03	0.03	0.05
25	Week 28–32.	1.56	0.06	−0.58	0.06
64	Week 32–36.	1.41	0.04	0.4	0.06

$n_{\text{premature}} = 104$.
Significant difference: $P < 0.05$.

32–36, whereas the proapoptotic Bax gene demonstrated significant overexpression in premature births at weeks 28–32 and 32–36, and no change of activity in premature births at weeks 24–28, compared to similar values of control samples (Table 6).

The expression of the antiapoptotic Bcl-2 gene in uterine fibroid tissue samples was shown to be significantly increased ($P < 0.05$) compared to expression values of samples in the control group, whereas the expression of the proapoptotic Bax gene did not show any significant difference (Table 7).

When comparing Bcl-2 and Bax gene expression in cases with negative and positive family histories of uterine fibroids, significant difference in gene expression activity was not identified.

Significant Bax gene expression difference was not detected according to the number of fibroids compared to physiological myometrium; however, Bcl-2 gene activity showed a significant correlation with the number of tumours ($P < 0.05$), i.e. overexpression was found to be more pronounced in cases of multiple fibroids (Table 8).

When assessing Bcl-2 and Bax gene expression activity in relation to the length of lactation period following pregnancies prior to the development of uterine fibroids, no significant changes were detected compared to the gene activity in women who had not lactated previously, whereas Bcl-2 gene activity was shown to be higher in the case of a shorter lactation period.

Table 7. Bax- and Bcl-2 gene expression activity in uterine fibroid tissue compared to the normal myometrium control group

Name of gene	$\Delta Ct_{\text{normal}} \pm \text{SE}^{(\text{I.})}$	$\Delta Ct_{\text{fibroid}} \pm \text{SE}^{(\text{II.})}$	α value $\pm \text{SE}(\alpha)^{(\text{III.})}$	Ln 2^{α}	LCL	UCL	*P*	Gene expression changes
Bcl-2	8.24 ± 0.83	6.03 ± 0.82	2.21 ± 0.75	1.53	0.2	3.24	0.04	Overactivity
Bax	12.72 ± 1.01	13.93 ± 0.9	−1.21 ± 0.95	−0.83	−1.24	1.77	0.06	Not changed in function

UCL: upper confidence limit.
LCL: lower confidence limit.
Significant difference: $P < 0.05$.
(I.) $\Delta Ct_{\text{lnormal}} = Ct_{\text{assesed gene}} - Ct_{\beta\text{-actine}}$.
(II.) $\Delta Ct_{\text{fibroid}} = Ct_{\text{assesed gene}} - Ct_{\beta\text{-actine}}$.
(III.) $\alpha = \Delta Ct_{\text{normal}} - \Delta Ct_{\text{fibroid}}$.

Table 8. The Bcl-2 and Bax gene expression activity in relation to the number of fibroids compared to physiological myometrium

Number of fibroids	α value ± SE(α)	Ln 2^{α}	*P*	Gene expression changes
*Bcl-2*1 (n = 53)	2.01 ± 0.69	1.13	0.04	Overactivity
*Bcl-2*2 (n = 13)	1.89 ± 0.8	1.37	0.04	Overactivity
*Bcl-2*more than 2 (n = 23)	2.3 ± 0.74	1.69	0.04	Overactivity
*Bax*1 (n = 53)	−0.65 ± 0.98	−0.45	0.06	Not changed in function
*Bax*2 (n = 13)	0.02 ± 0.8	0.01	0.05	Not changed in function
*Bax*more than 2 (n = 23)	−0.42 ± 0.64	−0.29	0.05	Not changed in function

$\alpha = \Delta Ct_{\text{normal}} - \Delta Ct_{\text{fibroid.}}$
Control gene: *β-aktin*.

DISCUSSION

The clinical results obtained are rather referential and serve as adequate clinical characterisation of the assessed patient population, as – based on the low number of cases – it has no statistical value.

Among environmental factors, the most important etiological factor of premature delivery was shown to be smoking during pregnancy. Smoking decreases the amount of available oxygen needed for intrauterine foetal development, and also decreases the level of serum IGF-1. The latter may also play a role in the imbalance of energy intake – oxygenation axis which is essential for the intrauterine health of the foetus [10].

Confirming ample literature data, our research also showed an inverse ratio between the number of pregnancies and incidence of uterine fibroids [36].

Regarding lactation and incidence of uterine fibroids, our results have shown that women treated for uterine fibroid had been pregnant fewer times and for shorter periods of time than patients in the control group; therefore, the rate of non-lactating women in the group of uterine fibroids was found to be significantly higher (27.4% vs 3%). Prolactin, the hormone regulating the lactation period, stimulates rather than inhibits the development of fibroids [33].

Embedding of placental tissue into the uterine wall is an aggressive process from a histological aspect, which brings the growth of malignant tumours into mind to some degree. Genes regulating apoptosis can be identified during the entire gestational period [24]. Although some studies reported an overexpression of the antiapoptotic Bcl-2 gene at the end of pregnancy [5], in the case of eutrophic intrauterine foetal development, significantly more data are available confirming underactivity [2, 32]. The expression of the proapoptotic Bax gene during physiological pregnancy probably increases near to the end of pregnancy, or at least that is being suggested by literature data [19, 45].

Based on assessments on placenta samples from pregnancies with intrauterine growth restriction, Barrio and his group confirmed decreased expression of the antiapoptotic Bcl-2 gene, whereas Agata and colleagues attributed the apoptotic imbalance to the overexpression of the proapoptotic Bax gene, and found placental Bcl-2 gene expression to be unchanged [2, 25]. Our study data match the results of Barrio et al., i.e. we could confirm for a large number of cases that in case of intrauterine growth restriction, a decrease in placental Bcl-2 gene activity can be observed [29] resulting in a less dominating presence of apoptosis-inhibiting mechanisms [2]. In pregnancies with intrauterine growth restriction, Agata et al. found an increase in placental Bax gene expression activity which stimulates the process of programmed cell death [25], and this

was also confirmed by Heazell et al.'s studies [21]. Unlike these previous results, our analysis did not show any difference in placental Bax gene activity in cases of intrauterine growth restriction compared to the control group.

De Falco et al. recommends the simultaneous assessment of Bcl-2 and Bax gene expression activities during physiological pregnancies for a flexible interpretation of the phenomenon of apoptosis [9]. This model allows simultaneous review of the changes in the activity of the most important proapoptotic and antiapoptotic genes, also highlighting the fact that one cannot interpreted without the other. Due to the placental functional disorders present in pathological pregnancies (such as IUGR) this model is even more useful, as there is a higher chance that pathological conditions are associated with changes in the regulation of programmed cell death. Based on the above model, our study results match the hypothesis suggesting changes in the activity of genes that exert stimulating and inhibitory effect on the process of apoptosis in the case of IUGR. However, gene expression observed in our study confirmed a decrease in antiapoptotic effects in the background of IUGR rather than an increase in gene activity stimulating programmed cell death.

The expression pattern of the assessed genes (Bcl-2, Bax) that influence programmed cell death did not show any difference in relation to the degree of underdevelopment of the foetus with intrauterine growth restriction [3].

Several studies investigated the role of apoptosis in the development of the premature rupture of the membranes, which is the most common cause for premature birth [40]. Fortunato et al. detected a definitive increase in Bax and a decrease in Bcl-2 gene activity in placental samples from premature births [15]. We also observed an undeniable increase in the activity of the proapoptotic Bax gene in placenta samples from premature births; however, we did not detect any changes in gene expression regarding the antiapoptotic Bcl-2 gene; i.e. in the process of apoptosis contributing to the initiation of premature birth, the overexpression of the activating Bax gene might play the primary role, and the underactivity of the inhibitory Bcl-2 gene is less important.

Gestational age at the time of premature birth is a relevant aspect regarding the placental activity of the proapoptotic Bax gene, as it shows an increase in activity in premature births after the 28th gestational week, whereas no change at all was identified in the activity of the Bcl-2 gene. Apoptosis might play a less important part in premature births between gestational weeks 24–28 than in cases following the 28th gestational week.

In both benign and malignant tumour growth, an imbalance between cell division and death can always be observed. According to our results, the Bcl-2 gene is overexpressed in uterine leiomyomas compared to the gene expression pattern in the normal myometrium, indicating that the imbalance of apoptosis playing a role in the development of fibroids may be attributed to the overactivity of an antiapoptotic gene (Bcl-2), while the proapoptotic Bax gene is functioning normally [7].

The gene expression analysis of apoptosis in case of the most common OB/GYN diseases may contribute to successful research for the efficient prediction of IUGR, premature birth as well as uterine fibroids in the future.

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