

Alteration of apoptosis-related genes in postmenopausal women with uterine prolapse

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Abstract

Introduction and hypothesis We aimed to compare expression levels of antiapoptotic and proapoptotic genes in parametrial and vaginal tissues from postmenopausal women with and without pelvic organ prolapse (POP). We hypothesized that the expression of genes that induce apoptosis may be altered in vaginal and parametrial tissues in postmenopausal women with POP.

Methods Samples of vaginal and parametrial tissues were obtained from postmenopausal women with ($n=10$) and without ($n=10$) POP who underwent vaginal or abdominal hysterectomy. Expression levels of antiapoptotic (*BCL-2*, *BCL-XL*) and proapoptotic (*BAX*, *BAD*) genes were studied by real-time reverse-transcription polymerase chain reaction (RT-PCR).

Results Gene expression levels of *BCL-2* ($P<0.001$), *BCL-XL* ($P<0.001$), *BAX* ($p=0.001$), and *BAD* ($p=0.004$) were all higher in vaginal tissues from the POP group compared with the non-POP group. Similarly, gene expression levels of *BCL-2* ($p<0.001$), *BCL-XL* ($p<0.001$), *BAX* ($p<0.001$), and *BAD* ($p<0.001$) in parametrial tissues were also significantly higher in the POP group compared with the non-POP group. Additionally, expression levels of *BCL-2* ($p=0.05$), *BCL-XL* ($p<0.05$), *BAX* ($p=0.05$), and *BAD* ($p=0.07$) in the POP group were higher in parametrial tissue than in vaginal tissue samples.

Conclusions Antiapoptotic and proapoptotic gene expression levels differed significantly between postmenopausal women with and without POP. Bcl-2 family genes were overexpressed in the parametrium of patients with POP compared with

vaginal tissue, suggesting that the processes responsible for POP have a greater effect on parametrial tissue than vaginal tissue during the development of POP.

Keywords Apoptosis · Bcl-2 family gene · Pelvic organ prolapse

Introduction

Pelvic organ prolapse (POP) is the downward descent of pelvic organs resulting in protrusion of the vagina, uterus, or both. It can affect the anterior and posterior walls of the vagina or uterus or the apex of the vagina, usually in some combination [1]. The pelvic organs are supported by both the pelvic floor muscles and their intact attachments to the endopelvic fascia, and damage to this compartment leads to the development of POP [2]. The condition is more common in elderly and multiparous women, and parity is considered to be one of the main risk factors [3]. However, POP does not occur in some women with risk factors but does occur in some with no obvious risk factors. This indicates the involvement of predisposing factors other than the known risk factors for POP development. Various types of abnormalities have been documented in the smooth muscle and connective tissue of the vaginal wall in women with POP, including increased proteolytic activity and apoptotic index in the extracellular matrix [4] and increased apoptosis in the anterior vaginal wall [5]. However, biochemical differences in the vagina and parametrium between women with and without POP are not always evident [6]. Apoptosis is a noninflammatory mechanism of cell death. The mitochondrial cell-death pathway is one of the major pathways responsible for apoptosis, involving the Bcl-2 family proteins, including Bcl-2, Bcl-xl, Bax, and Bad. In this pathway, Bcl-2 and Bcl-xl proteins are antiapoptotic, whereas Bax and Bad proteins are proapoptotic. The balance between

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antiapoptotic and proapoptotic genes is therefore critical in determining the ultimate apoptotic rate; the greater the expression level of proapoptotic genes, the more likely the cell will die [7].

We hypothesized that the expression levels of genes that induce apoptosis may be altered in vaginal and parametrial tissues in postmenopausal women with compared with postmenopausal women without POP. In this study, we therefore compared expression levels of antiapoptotic and proapoptotic genes in parametrial and vaginal tissues from postmenopausal women with and without POP.

Materials and methods

Twenty consecutive postmenopausal patients attending the Obstetric and Gynecology Clinic of the Medicine School of Dokuz Eylul University, İzmir, and undergoing abdominal or vaginal hysterectomy for benign gynecological disorders, were selected for the study. The indication for hysterectomy in the non-POP group was benign adnexal cysts. Patients were divided into two groups: a POP group, with uterine prolapse stage III or IV ($n=10$), and a control group, without uterine prolapse (stage 0) ($n=10$). Written informed consent was obtained from all patients, and the study was approved by the Institutional Review Board. All individuals in both groups underwent assessment of POP stage based on the International Pelvic Organ Prolapse Quantification (POP-Q) system, a subjective assessment for POP provided by the International Continence Society (ICS) [8]. Examinations were performed with the patient in the supine position, and the Valsalva maneuver or coughing was used to demonstrate maximum prolapse descent. Patients were excluded if they had gynecological cancer, cervical intraepithelial lesions, endometrial hyperplasia, connective tissue disorders, chronic disabling disorders, alcoholism, a smoking habit, or who had used hormonal replacement therapy. Total abdominal hysterectomy with bilateral salpingo oophorectomy was performed in the control group, and vaginal hysterectomy with anterior–posterior colporrhaphy was performed in the POP group. Parametrial biopsies were obtained from the parametrium (cardinal ligament) that was not excised with the uterus during abdominal hysterectomy and were taken about 0.5–1 cm lateral to the cervix, starting from the proximal to the distal edge of the parametrial tissue between the Heaney clamp and the uterine cervix. In the POP group, full-thickness vaginal biopsies were obtained from the anterior corpectomy side during vaginal hysterectomy. In the control group, vaginal biopsies were obtained as full-thickness biopsies from the apex of the anterior vaginal wall after total hysterectomy. Vaginal and parametrial samples in the POP group were taken using Metzenbaum scissors. Parametrial samples in the non-POP group were taken using a size 10 scalpel, and vaginal samples

were taken using Metzenbaum scissors. All vaginal tissue samples were and assessed by histopathology.

Total RNA extraction and cDNA synthesis

Tissue samples were put into 5-ml sterile tubes containing RNA stabilization and protection solution (RNAlater; Qiagen, Hamburg, Germany). After transfer to the laboratory, total RNA (tRNA) was extracted using a High Pure RNA Isolation Kit according to the manufacturer's protocol (Roche Diagnostics). Complementary DNA (cDNA) was synthesized from 2 μ g of tRNA using a SuperScript First-Strand Synthesis System for real-time reverse-transcription polymerase chain reaction (RT-PCR), according to the manufacturer's protocol (Invitrogen, CA, USA). The mixture was incubated at 42 °C for 50 min, then 72 °C for 15 min. After the addition of 2 U RNase H, PCR was performed in a volume of 20 μ l containing 2 μ l cDNA.

Real-time RT-PCR

Real-time RT-PCR was carried out using a Light Cycler 2.0 (Roche Diagnostics) instrument and Light Cycler FastStart DNA MasterPLUS SYBR Green I (Roche Diagnostics) kit. Reactions were performed in a 20- μ l volume with 5 pmol of each primer and 2 μ l of cDNA template derived from reverse-transcribed RNA from the tissue. The hypoxanthine phosphoribosyl-transferase (HPRT) housekeeping gene was used as an endogenous control and reference gene for relative quantifications. The sequences of the oligonucleotide primers were as follows:

HPRT (F) 5'-GTGGAGATGATCTCTCAACT-3' and HPRT (R) 5'-ACATGATTCAAATCCCTGAAG-3'; *BAX* (F) 5'-AAGAAGCTGAGCGAGT-3' and *BAX* (R) 5'-GCCCATGATGGTTCTG-3'; *BCL-XL* (F) 5'-GCTGGTGGTTGACTTTC-3' and *BCL-XL* (R) 5'-GGATGGGTTGCCATTGA-3'; *BCL-2*(F) 5'-TCAGGCTGCTTGGGATAAAGATG-3' and *BCL-2*(R) 5'-GGCTTCTGGAGGACATTTGGA-3'; and

Table 1 Clinical characteristics of women with and without uterine prolapse

	POP group ($n=110$) median (range)	Non-POP group ($n=10$) median (range)	<i>P</i> value
Age	64.5 (51–73)	57 (45–68)	0.07*
Gravidity	3 (1 8)	2 (1 6)	NS
Parity	3 (1 7)	2 (1 4)	0.03*
BMI	27 (24.2–33.5)	29.8 (25.3–32.5)	NS
Menopause age	48.5 (40–53)	49.5 (43–55)	NS
POP stage	3 (3 4)	0	<0.001*

POP pelvic organ prolapse, BMI body mass index

*Statistically significant

Table 2 Expression levels of antiapoptotic (*BCL-2* and *BCL-XL*) and proapoptotic (*BAX*, *BAD*) Bcl-2 family members in women with and without pelvic organ prolapse (POP)

Gene	Vaginal tissue			Parametrial tissue		
	POP group (n=10) median (range)	Non-POP group (n=10) median (range)	P value	POP group (n=10) median (range)	Non-POP group (n=10) median (range)	P value
<i>BCL-2</i>	6.0 (3.2–9.5)	1.4 (0.7–2.2)	<0.001	19.5 (12.3–35.9)	2.2 (1.2–3.4)	<0.001
<i>BCL-XL</i>	7.2 (4.5–13.3)	1.6 (0.9–3.1)	<0.001	15.6 (8.6–25.7)	1.5 (0.8–2.9)	<0.001
<i>BAX</i>	0.9 (0.5–1.9)	0.4 (0.2–0.8)	0.001	3.0 (1.2–8.5)	0.6 (0.2–1.5)	<0.001
<i>BAD</i>	1.3 (0.4–3.5)	0.4 (0.1–1.2)	0.004	2.8 (1.2–5.2)	0.4 (0.2–1.0)	<0.001

BAD (F) 5'-GAGGTCCTGAGCCGACAG-3' and *BAD* (R) 5'-CTTCCTCTCCCACCGTAGC-3'. Primers were synthesized commercially by Genosys Biotechnology Inc. Forty cycles were performed. HPRT was used as an endogenous reference against which the different template values (*BCL-2*, *BCL-XL*, *BAD*, and *BAX*) were normalized. The threshold cycle (Ct) method was used for quantification. The relative *BCL-2*, *BCL-XL*, *BAD*, and *BAX* gene expression levels corrected for expression of the normalizer gene (HPRT1) were divided by one normalized control sample value (calibrator sample) to generate the relative quantification. Calculations were performed using Light Cycler 2.0 software.

Statistical analysis

Results are expressed as median or mean \pm standard deviation (SD). Comparisons between groups and within groups were performed using the Mann–Whitney *U* test or Wilcoxon's signed-rank test. Differences were considered significant at $P < 0.05$. All statistical calculations were performed using SPSS 15.0 for Windows.

Results

Parametrial and vaginal biopsies were obtained from postmenopausal patients with ($n=10$) and without ($n=10$) POP

who underwent hysterectomy with no complications. There were no significant differences in body mass index (BMI), menopause age, smoking habit, and gravidity between groups, though median age and parity were higher in the POP group; clinical characteristics are given in Table 1. The stage of uterine prolapse was significantly higher in the POP group (mean 4, range 3–4) compared with the non-POP group (mean 0, range 0) ($p=0.002$). POP-Q points for C, Ba, Bp, and D were noted for patients in the POP group. Measurement ranges for Ba, Bp, C, and D in the six patients with stage 3 prolapse were Ba (2–3), Bp (3–4), C (4–5), and D (1–2). Equivalent ranges in the four patients with stage 4 prolapse were Ba (4–5), Bp (4–5), C (7–8), and D (4–5).

Gene expression levels in tissues

Gene expression levels of *BCL-2* ($P < 0.001$), *BCL-XL* ($P < 0.001$), *BAX* ($p=0.001$), and *BAD* ($p=0.004$) in vaginal tissues in the POP group ($n=10$) were all higher than those in the non-POP group ($n=10$). Similarly, gene expression levels of *BCL-2* ($P < 0.001$), *BCL-XL* ($P < 0.001$), *BAX* ($P < 0.001$), and *BAD* ($P < 0.001$) in parametrial tissues in the POP group ($n=10$) were also significantly higher than those in the non-POP group ($n=10$) (Table 2). We also analyzed the ratio of antiapoptotic to proapoptotic genes. The *BCL-2/BAX*, *BCL-2/BAD*, *BCL-XL/BAX*, and *BCL-XL/BAD* ratios in vaginal tissue were higher in the POP group compared with the non-POP group, but the difference was only significant for the *BCL-2/*

Table 3 Ratios of antiapoptotic/proapoptotic gene expression levels in vaginal and parametrial tissues in women with and without pelvic organ prolapse (POP)

Gene	Vaginal tissue			Parametrial tissue		
	POP group (n=10) median(range)	Non-POP group (n=10) median(range)	P value	POP group (n=10) median(range)	Non-POP group (n=10) median(range)	P value
<i>BCL-2/BAX</i>	5.3 (4.1–11.8)	3.2 (2.6–5.1)	0.002*	9.3 (1.9–20.6)	3.0 (1.1–15.6)	0.082
<i>BCL-2/BAD</i>	4.2 (2.0–12.7)	2.6 (1.8–6.7)	0.059	8.0 (2.7–29.2)	4.2 (1.4–9.1)	0.096
<i>BCL-XL/BAX</i>	9.2 (2.7–23.5)	4.0 (1.5–12.4)	0.070	4.5 (2.2–12.9)	2.2 (0.8–7.5)	0.059
<i>BCL-XL/BAD</i>	8.1 (1.7–20.0)	2.8– (1.1–17.2)	0.199	5.0 (3.3–14.8)	3.1 (1.6–6.3)	0.004*

*Statistically significant

Table 4 Comparison of real-time reverse-transcriptase polymerase chain reaction (RT-PCR) results for Bcl-2 family members (*BCL-2* and *BCL-XL* antiapoptotic; *BAX*, *BAD* proapoptotic) within groups with regard to tissue type (vaginal vs parametrial tissue)

Gene	POP group (n=10)			Non-POP group (n=10)		
	Vaginal tissue median (range)	Parametrial tissue median (range)	P value	Vaginal tissue median (range)	Parametrial tissue median (range)	P value
<i>BCL-2</i>	6.0 (3.2–9.5)	19.5 (12.3–35.9)	0.005*	1.4 (0.7–2.2)	2.2 (1.2–3.4)	0.074
<i>BCL-XL</i>	7.2 (4.5–13.3)	15.6 (8.6–25.7)	0.005*	1.6 (0.9–3.1)	1.5 (0.8–2.9)	0.722
<i>BAX</i>	0.9 (0.5–1.9)	3.0 (1.2–8.5)	0.005*	0.4 (0.2–0.8)	0.6 (0.2–1.5)	0.022
<i>BAD</i>	1.3 (0.4–3.5)	2.8 (1.2–5.2)	0.007*	0.4 (0.1–1.2)	0.4 (0.2–1.0)	0.959

POP pelvic organ prolapse

*Statistically significant

BAX ratio ($p=0.002$). The same ratios were also higher in parametrial tissue in the POP group, but the difference was only significant for the *BCL-XL/BAD* ratio ($p=0.004$) (Table 3).

We also examined differences in apoptosis-related gene expression levels between vaginal and parametrial tissues within the same group of patients. The expression levels of *BCL-2* ($p=0.05$), *BCL-XL* ($P<0.05$), *BAX* ($p=0.05$), and *BAD* ($p=0.07$) in the POP group were significantly higher in parametrial tissue compared with vaginal tissue samples (Table 4). In contrast, the expression levels of *BCL-2*, *BCL-XL*, and *BAD* were similar in both tissues in the non-POP group, and only *BAX* expression was significantly higher in parametrial tissue compared with vaginal tissue samples ($p=0.022$).

Discussion

In this pilot study, we examined the expression of antiapoptotic and proapoptotic Bcl family members in vaginal and parametrial tissues from postmenopausal patients with or without POP. Tissue samples from the parametrium and anterior vagina were analyzed to determine which was more

affected by the processes leading to POP. Expression levels of both proapoptotic and antiapoptotic genes were increased in POP patients compared with non-POP patients (Fig. 1), but the increase was much more significant for antiapoptotic proteins and more pronounced in parametrial than in vaginal tissue (Figs. 2, 3). These results indicate that the levels of apoptosis-related proteins are altered in postmenopausal women with POP, especially in parametrial tissues. We suggest that cell turnover is increased during POP and that a tendency toward cell survival occurs to balance the increase in cell death. Antiapoptotic genes are thus more highly expressed than proapoptotic genes to maintain the tissue integrity.

Takacs et al. compared 12 women undergoing surgery for either POP repair or benign gynecological conditions and found that anterior vaginal wall prolapse was associated with a significant reduction in the proportion of smooth muscle area [5]. They also found a significant increase in apoptotic smooth muscle cells. In another study, the same authors compared the smooth muscle content and apoptotic cells in the uterosacral ligaments of women with and without POP and again found that apoptosis was increased in women with POP [4]. Both these studies used the terminal deoxynucleotidyl

Fig. 1 Bcl-2 family gene expression levels [real-time reverse-transcriptase polymerase chain reaction (RT-PCR) in vaginal tissues from patients with and without prolapse [mean \pm standard deviation (SD)]. All gene expression levels differed significantly between groups with and without pelvic organ prolapse. *BCL-2* and *BCL-XL* ($p<0.001$), *BAX* ($p=0.001$), *BAD* ($p=0.04$)

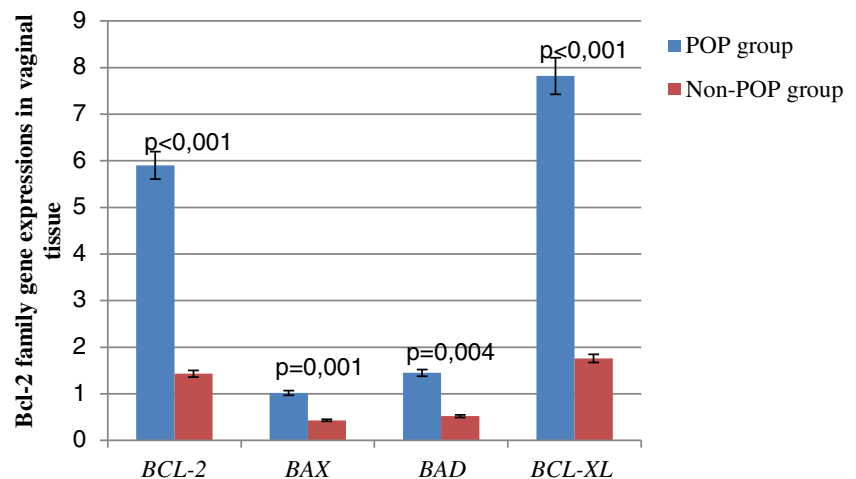
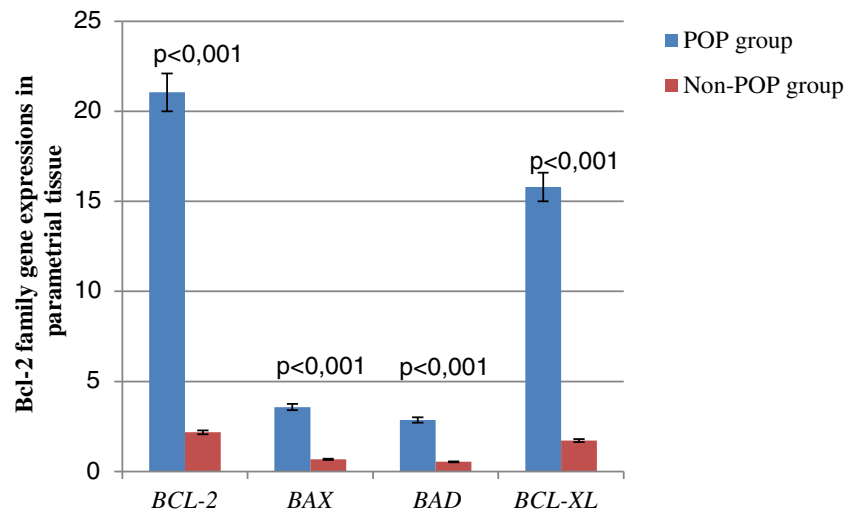


Fig. 2 Bcl-2 family gene expression levels [real-time reverse-transcriptase polymerase chain reaction (RT-PCR)] in parametrial tissues from patients with and without pelvic organ prolapse (POP) [mean \pm standard deviation (SD)]. All gene expression levels differed significantly between groups with and without POP. *BCL-2*, *BCL-XL*, *BAX*, *BAD* ($p < 0.001$)

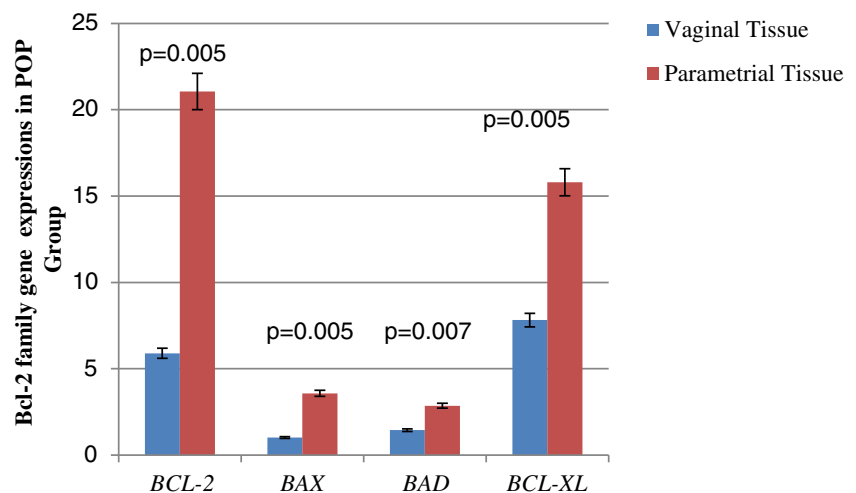


transferase-mediated 2'-deoxyuridine 5'-triphosphate (dUTP) nick-end-labelling (TUNEL) method to detect apoptotic cells, which defines the apoptotic index on the basis of TUNEL-stained cells and therefore only includes cells that have already undergone apoptosis. Neither of these studies measured Bcl-2 family expression, which indicates cellular sensitivity to apoptosis. In addition, trauma and hormonal deprivation, as well as triggering mechanisms other than the mitochondrial-cell-death pathway (Bcl-2 family proteins), might be responsible for apoptosis. In our study, expression levels of both the antiapoptotic genes *BCL-2* and *BCL-XL* and the proapoptotic genes *BAX* and *BAD* were all increased in vaginal and parametrial tissues in the POP group compared with the non-POP group. Furthermore, expression levels of all four antiapoptotic and proapoptotic genes in parametrial tissue were higher than those in vaginal tissue. These findings suggest that cell turnover is increased in vaginal and parametrial tissues in postmenopausal women with POP and that this cell

turnover is more pronounced in parametrial tissue. Given that the cardinal ligament (parametrium) conducts most of the pelvic loading forces, we suggest that it is the first and most severely affected supportive tissue when the POP process is triggered.

Expression of Bcl-2 family proteins in vaginal tissues from women with POP was examined by Wen et al. [9]. They studied 34 premenopausal women according to their menstrual phase (proliferative or secretory). Bcl-2 protein expression was only significantly decreased in women with POP during the secretory phase compared with controls [9]. The authors associated this finding with low estrogen and high progesterone levels in the secretory phase of menstruation, because estrogen down-regulates Bax [10] and progesterone decreases Bcl-2 expression [11]. They also recruited 12 postmenopausal women with mild ($n=6$) or severe ($n=6$) POP and found that only Bax and Bad expression levels were slightly higher in the severe POP group. These findings are not compatible with

Fig. 3 Comparison of Bcl-2 family gene expression levels [real-time reverse transcriptase polymerase chain reaction (RT-PCR)] between vaginal and parametrial tissues in the pelvic organ prolapse (POP) group [mean \pm standard deviation (SD)]. All gene expression levels differed significantly between vaginal and parametrial tissues in patients with POP. *BCL-2* vaginal vs. parametrial ($p < 0.005$), *BCL-XL* vaginal vs. parametrial ($p < 0.005$), *BAX* vaginal vs. parametrial ($p < 0.005$), *BAD* vaginal vs. parametrial ($p < 0.007$)



those of our study reported here; we found that both antiapoptotic and proapoptotic genes were overexpressed in the POP group compared with controls, with high antiapoptotic to proapoptotic gene expression ratios. A previous study investigating Bcl-2 family protein expression levels in cervical cancer patients found higher Bcl-2 levels in elderly and postmenopausal controls than in younger women, suggesting a biological difference in cervical tissue in relation to patient age [12].

Individual variations in the quality of ligaments and fasciae of the pelvic floor have been identified as essential factors in the genesis of POP. Some studies focused on the quality and quantity of collagen tissue in the parametrium in women with prolapse. Takano et al. showed that the amount of collagen was significantly lower in patients with uterine prolapse than in those without but found no differences between patients with and without prolapse when they were grouped according to premenopausal or postmenopausal status [13]. Another study evaluating the content and quality of type I collagen in the parametrium of women with and without uterine prolapse found that collagen fibers were shorter, thinner, and arranged in a more disorderly manner in patients with prolapse [14]. In our study, Bcl-2 family genes were overexpressed in the parametrium in patients with POP compared with vaginal tissue, suggesting that parametrial tissue is more susceptible than vaginal tissue to gene alterations in women with POP.

We observed higher expression levels of *BCL-2*, *BCL-XL*, *BAX*, and *BAD* genes in postmenopausal patients with POP compared with controls as well as overexpression of all four genes in parametrial tissue compared with vaginal tissue. As noted above, these findings contrast with those of Wen et al., who found higher expression levels of proapoptotic but lower levels of antiapoptotic proteins in patients with POP. This discrepancy may be associated with differences in age and menopausal status between the different patient groups. Hypoxia, trauma, and estrogen deprivation increases with increasing age, and apoptosis-related genes might be modulated within this time period in patients with POP. However, our findings and those of other studies suggest that antiapoptotic and proapoptotic gene modulation is likely to contribute to POP severity.

The limitations of our study are its small sample size and a study design that did not allow us to determine cause or effect. The age of women with POP was higher than those without; we therefore could not exclude a possible effect of age on the results can only comment on age-related changes. Although our sample size was small, restricting our study to postmenopausal patients eliminated the potential influence of hormones on gene expression. In addition, by analyzing both parametrial and vaginal tissues, we were able to determine tissue-specific gene expression levels within the POP group.

In summary, antiapoptotic and proapoptotic gene expression levels were significantly altered in postmenopausal

women with compared with those without POP. Furthermore, Bcl-2 family genes in POP patients were overexpressed in the parametrium compared with vaginal tissue, suggesting that parametrial tissue is more severely affected during the processes responsible for POP. These results suggest that Bcl-2-family gene expression is altered in women with POP and that this alteration is more serious in parametrial tissue. Further studies with larger sample sizes adjusted for age and menopausal status are needed to clarify the mechanisms responsible for POP in order to allow the development of preventive and therapeutic treatment options.

Conflicts of interest None

References

- Abrams P, Cardozo L, Fall M, Griffiths D, Rosier P, Ulmsten U, van Kerrebroeck P, Victor A, Wein A (2002) The standardisation of terminology of lower urinary tract function: report from the Standardisation Sub-committee of the International Continence Society. *Am J Obstet Gynecol* 187:116–126
- Brown JS, Waetjen LE, Subak LL, Thom DH, Van den Eeden S, Vittinghoff E (1997) Pelvic organ prolapse surgery in the United States. *Am J Obstet Gynecol* 186:712–716
- Mant J, Painter R, Vessey M (1997) Epidemiology of genital prolapse: observations from the Oxford Family Planning Association Study. *Br J Obstet Gynaecol* 104:579–585
- Takacs P, Nassiri M, Gualtieri M, Candiotti K, Medina CA (2009) Uterosacral ligament smooth muscle cell apoptosis is increased in women with uterine prolapse. *Reprod Sci* 16:447–452
- Takacs P, Gualtieri M, Nassiri M, Candiotti K, Medina CA (2008) Vaginal smooth muscle cell apoptosis is increased in women with pelvic organ prolapse. *Int Urogynecol J Pelvic Floor Dysfunct* 19:1559–1564
- Kati LM, Feldner PC, de Castro RA, Kobayashi EY, Sartori MG, Nader HB, Castello Girão MJ (2010) Analysis of glycosaminoglycans in the parametrium and vaginal apex of women with and without uterine prolapse. *J Women's Health (Larchmt)* 19:1341–1344
- Danial NN, Korsmeyer SJ (2004) Cell death: critical control points. *Cell* 116(2):205–219
- Bump RC, Mattiasson A, Bø K, Brubaker LP, DeLancey JO, Klarskov P, Shull BL, Smith AR (1996) The standardization of terminology of female pelvic organ prolapse and pelvic floor dysfunction. *Am J Obstet Gynecol* 175:10–17
- Wen Y, Ho JY, Polan ML, Chen B (2011) Expression of apoptotic factors in vaginal tissues from women with urogenital prolapse. *Neurourol Urodyn* 30:1627–1632
- Tsukahara S, Hojo R, Kuroda Y, Fujimaki H (2008) Estrogen modulates Bcl-2 family protein expression in the sexually dimorphic nucleus of the preoptic area of postnatal rats. *Neurosci Lett* 432:58–63
- Gompel A, Sabourin JC, Martin A, Yaneva H, Audouin J, Decroix Y, Poitout P (1994) Bcl-2 expression in normal endometrium during the menstrual cycle. *Am J Pathol* 144:1195–1202
- Ferrandina G, Mozzetti S, Marone M, Fagotti A, Macchia G, Mancuso S, Scambia G (2000) Bcl-2, bax, bcl-x(L) and bcl-x(S) expression in neoplastic and normal cervical tissue. *Cancer Lett* 155:19–27

13. Takano CC, Girão MJ, Sartori MG, Castro RA, Arruda RM, Simões MJ, Baracat EC, Rodrigues de Lima G (2002) Analysis of collagen in parametrium and vaginal apex of women with and without uterine prolapse. *Int Urogynecol J Pelvic Floor Dysfunct* 13:342–345
14. Barbiero EC, Sartori MG, Girão MJ, Baracat EC, de Lima GR (2003) Analysis of type I collagen in the parametrium of women with and without uterine prolapse, according to hormonal status. *Int Urogynecol J Pelvic Floor Dysfunct* 14:331–334