의학석사 학위논문

Poly(ADP-ribose) polymerase-1 gene polymorphism as a determinant of individual susceptibility to pelvic organ prolapse

골반장기탈출증과 Poly(ADP-ribose) polymerase-1 유전자 다형성에 관한 연구

2013년 2월

서울대학교 대학원

의학과 임상의과학과 전공

김 지 영

Poly(ADP-ribose) polymerase-1 gene polymorphism as a determinant of individual susceptibility to pelvic organ prolapse

지도교수 전 명 재

이 논문을 의학석사 학위논문으로 제출함

2012년 10월

서울대학교 대학원

의학과 임상의과학과 전공

김 지 영

김지영의 의학석사 학위논문을 인준함

2013년 1월

위 원 장	김 석 현	(인)
부위원장	전 명 재	(인)
위 원	김 재 원	(인)

Abstract

Ji Young, Kim

College of Medicine

Department of Clinical Medical Sciences

The Graduate School

Seoul National University

Introduction: Apoptotic cell death, likely induced by oxidative stress, contributes to the development of pelvic organ prolapse (POP). Because poly(ADP-ribose) polymerase-1 (PARP-1) is an important mediator of the cellular response to oxidative stress, genetic variations in the PARP-1 gene may play a role in the pathogenesis of POP. This study aimed to determine the association between POP and the Val762Ala polymorphism in the PARP-1 gene.

Methods: A total of 370 women were enrolled in the study. The patient group consisted of 215 women diagnosed with POP stage II or greater, whereas the control group consisted of 155 postmenopausal women with POP stage 0 or I who visited the hospital for treatment of benign gynecologic disease or a routine gynecologic checkup. Genomic DNA was extracted from peripheral blood samples with a DNA purification kit, and the PARP-1 Val762Ala polymorphism was genotyped by real-time PCR analysis using a TaqMan assay.

Results: The genotype distribution in the patient group was significantly different from that of the control group (TT/TC/CC rates were 33.5%/51.6%/14.9% and 29.0%/45.8%/25.2% for the POP and control groups, respectively; p=0.046). Furthermore, the C allele frequency was significantly lower in the patient group than in the controls (40.7% vs. 48.1%; p=0.046). Women with the CC genotype had a 0.513-fold lower risk of developing POP (95% confidence interval [CI], 0.282-0.934; p=0.029) than women with the TT genotype, and women with the C allele had a 0.742-fold lower risk of developing POP than women with the T allele (95% CI, 0.553-0.997; p=0.047). Moreover, when the data were re-analyzed excluding the women with mild POP (POP stage II), these observations were more prominent.

Conclusions: These findings suggest that the PARP-1 Val762Ala polymorphism reduces the risk of POP.

Keywords: Pelvic organ prolapse, Oxidative stress, Polymorphism, PAPR-1

Student Number: 2011-21976

Contents

List of Tables

List of Figures

Introduction

Pelvic organ prolapse (POP) is a major health problem in adult women, causing bladder, bowel, and pelvic symptoms that can have an adverse effect on their daily activities and quality of life (1). POP affects almost half of all women over the age of 50 (2), and because the population is aging, the demand for POP care is estimated to double over the next 40 years (3). Also, Korea is entering the aging society faster than Western countries (4)(Figure 1). Thus, prevention of POP is important to reduce the socioeconomic burden. The etiology of POP is multifactorial; in addition to advancing age, vaginal parity, and obesity, a genetic predisposition for POP may influence the development of POP (5). The Swedish Twin Registry Study demonstrated that genetic factors contribute to the development of POP to the same extent as environmental factors (6). Therefore, identifying women at risk for POP by screening for genetic variations may help improve preventive strategies.

POP is caused by a loss of support resulting from biomechanical weakness of the pelvic supportive tissues. One of the typical findings in the pelvic supportive connective tissues of women with POP is increased apoptotic cell death (7,8). Apoptosis in these tissues leads to decreases in fibroblasts and smooth muscle cells and their products (collagen, elastin, and smooth muscle). Recently, Kim et al (9) found that the increased apoptosis in the uterosacral ligaments of POP patients is intimately linked to oxidative stress. Moreover, oxidative stress was present in measurable quantities even in patients with mild POP, where apoptotic cell death was not prominent, which implies that oxidative stress may initiate or propagate apoptotic cell death in the uterosacral ligaments.

DNA repair is essential for the maintenance of genome integrity. Poly(ADP-ribose) polymerase-1 (PARP-1) plays a critical role in base

excision repair (BER), a major pathway involved in the repair of oxidative DNA damage (10). PARP-1 recognizes a single-stranded DNA break and facilitates poly(ADP-ribosyl)ation of target proteins. It also recruits X-ray repair cross-complementing 1 (XRCC1), an important scaffold protein for BER that interacts with DNA polymerase II (polB), which fills the gap, and DNA ligase III, which completes the repair process (11). Additionally, PARP-1 acts as an important mediator of cell fate decisions (survival or death) according to the type, strength, and duration of the stress stimuli (12).

Given data from studies by Kim et al (9) and its biologic plausibility, genetic variations in the PARP-1 gene may influence individual susceptibility to POP. A number of polymorphisms in the PARP1 gene have been reported, but the Val762Ala polymorphism (rs1136410) has been studied most frequently because it is a nonsynonymous polymorphism that causes an amino acid change as well as a common polymorphism with a minor allele frequency of at least 5% (13). I hypothesized that this polymorphism may modulate the risk of POP. To test this idea, I determined the prevalence of the PARP-1 Val762Ala polymorphism in women with and without POP.

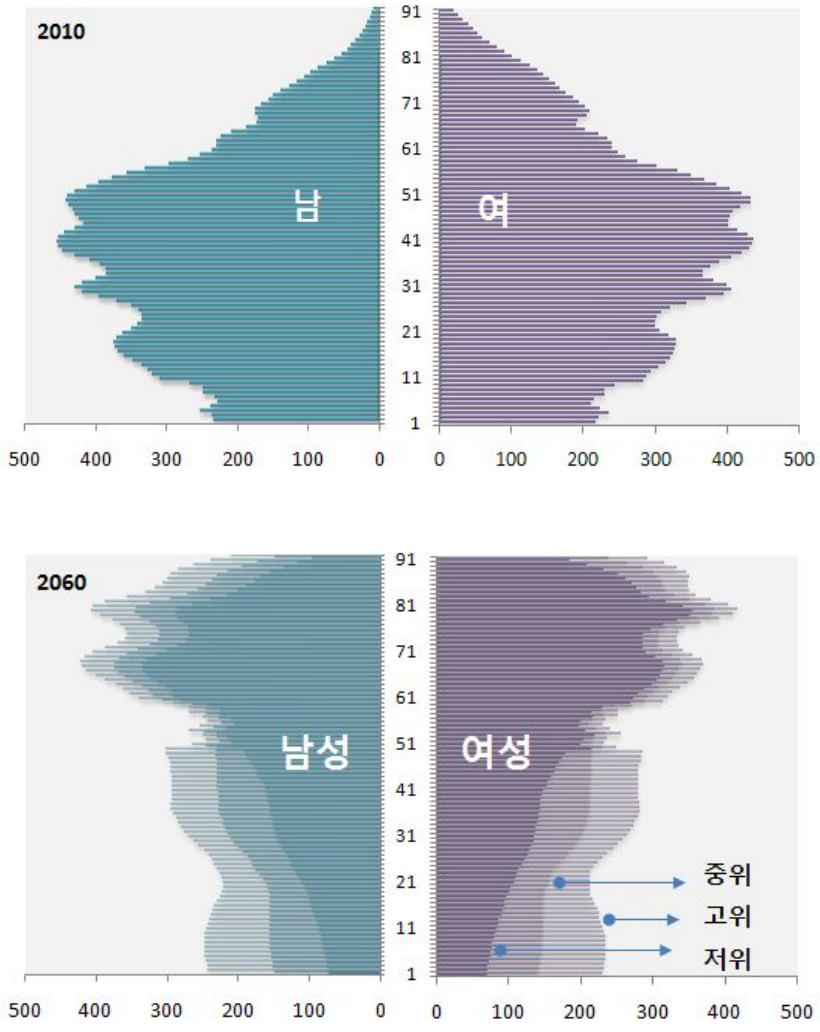

Figure 1. Chart of population distribution in 2010 and 2060 (expected) (14)

Materials and Methods

Subjects

A total of 370 Korean women were recruited prospectively and consecutively from the Department of Obstetrics and Gynecology, Seoul National University Hospital, Korea, from March 2009 to November 2011. All participants were examined according to the International Continence Society's Pelvic Organ Prolapse Quantification (POP-Q) system. The patient group consisted of 215 women who were diagnosed with POP stage II or greater. The control group consisted of 155 postmenopausal women diagnosed with POP stage 0 or I who visited the hospital for treatment of benign gynecologic disease or a routine gynecologic checkup. Women with endometriosis, leiomyoma, adenomyosis, and malignancy were excluded from the control group. Women who had received local or systemic hormonal therapy or antioxidants were excluded from both groups. Peripheral blood was obtained from participants in each group. The study was approved by the review board for human research at Seoul National University Hospital (H-1205-060-410), and informed consent was obtained from each woman.

Clinical variables such as age, vaginal parity, body mass index (BMI), menopause, and history of previous hysterectomy were recorded. Menopause was defined as the cessation of menses for at least one year.

Genotyping of the Val762Ala polymorphism

Genomic DNA was isolated and extracted from whole blood with a QIAamp DNA Blood Mini kit (Qiagen, Hilden, Germany). Genotyping for the PARP-1 Val762Ala polymorphism (rs1136410) was carried out using a Custom TaqMan SNP Genotyping Assay and analyzed on an ABI Prism 7500

Sequence Detection System (Applied Biosystems, Foster City, CA, USA). Each reaction contained 10 μl of the 2X TaqMan Genotyping Master Mix and 25 ng of DNA (assay-on-demand number: C_1515368_1_). The PCR cycling conditions consisted of a 1-minute cycle at 60°C and a 10-minute cycle at 95°C, followed by 50 cycles at 95°C for 15 seconds and 60°C for 90 seconds. Nuclease-free water was used as a negative PCR control for each amplification. For quality control, the genotyping analysis was performed blind with respect to case/control status.

Statistical analysis

The number of subjects was initially chosen to detect a difference in overall risk for POP related to the PARP-1 Val762Ala polymorphism with an effect size of 0.145, a power of 0.8, and type I error of 5%, following Jin *et al.* (15). According to G*Power 3.0, at least 153 women had to be included in each group.

All statistical analyses were performed using SPSS 19.0 for window (SPSS, Chicago, IL, USA). The genotypic frequencies for a single polymorphism of the PARP-1 Val762Ala gene were tested against Hardy-Weinberg equilibrium by the chi-square test. Allele and genotype frequencies of the single polymorphism were compared between the patient and control groups using the chi-square test. Adjusted odds ratios were calculated using a multivariable logistic regression model that controlled for BMI and included 95% confidence intervals (CIs). *P*-values of less than 0.05 were considered significant.

Results

Table 1 shows the clinical characteristics of the patients with POP and the controls. The median age, vaginal parity and history of previous hysterectomy were similar between the groups, but the BMI and menopause patterns were significantly different.

Genotyping of the PARP-1 Val762Ala polymorphism was successfully performed for all subjects. The genotype distribution in both groups followed Hardy-Weinberg equilibrium (p=0.59). However, the genotype distribution of the PARP-1 Val762Ala polymorphism in the patient group was significantly different from that of the control group (TT/TC/CC rates were 33.5%/51.6%/14.9% and 29.0%/45.8%/25.2% for the POP and control groups, respectively; p=0.046). The C allele frequency was significantly lower in the patients than in the controls (40.7% vs. 48.1%; p=0.046). Women with the CC genotype had a 0.513-fold lower risk of developing POP (95% CI, 0.282-0.934; p=0.029) than women with the TT genotype, and women with the C allele had a 0.742-fold lower risk of POP than women with the T allele (95% CI, 0.553-0.997; p=0.047) (Table 2). Morever, when the data were re-analyzed excluding the women with mild POP (POP stage II), these observations were more prominent. In advanced POP patients, C allele frequency was significantly lower than in the controls (39.7% vs. 48.1%; p=0.029). Moreover, women with the CC genotype had a 0.461-fold lower risk of developing advanced POP (95% CI, 0.245-0.870; p=0.017) than women with the TT genotype, and women with the C allele had a 0.716-fold lower risk of advanced POP than women with the T allele (95% CI, 0.527-0.973; p=0.033) (Table 3).

Table 1. Clinical characteristics of POP patients and controls recruited in this study

	Patients (n=215)	Controls (n=155)	P-value
Age (yr)	62.0 (14)	61.0 (10)	0.136
Vaginal parity	3 (1)	2 (2)	0.248
Body mass index (kg/m^2)	24.2 (3.6)	23.6 (3.5)	0.015
Menopause	185 (86.0)	155 (100)	<0.001
Previous hysterectomy	16 (7.4)	8 (5.2)	0.380
POP-Q stage, n (%)			<0.001
0-I	0	155 (100)	
II	30 (14.0)	0	
III	158 (73.5)	0	
IV	27 (12.6)	0	

Values are presented as median (interquartile range) or number (%)

Table 2. Distribution of the PARP-1 Val762Ala polymorphism in POP patients and controls

	Patients	Controls	P-value*	OR (95% CI)†
Genotype			0.046	
TT	72 (33.5)	45 (29.0)		Reference
TC	111 (51.6)	71 (45.8)		0.980 (0.608-1.581)
CC	32 (14.9)	39 (25.2)		0.513 (0.282-0.934)
Allele			0.046	
T	255 (59.3)	161 (51.9)		Reference
C	175 (40.7)	149 (48.1)		0.742 (0.553-0.997)

Values are presented as number (%)

*Evaluated by chi-square test

†Calculated by multivariable logistic regression including body mass index and PARP1 Val762Ala polymorphism

Table 3. Distribution of the PARP-1 Val762Ala polymorphism in advanced POP patients (n=185) and controls (n=155)

	Patients	Controls	P-value*	OR (95% CI)†
Genotype			0.024	
TT	63 (34.1)	45 (29.0)		Reference
TC	97 (52.4)	71 (45.8)		0.987 (0.604-1.615)
CC	25 (13.5)	39 (25.2)		0.461 (0.245-0.870)
Allele			0.029	
T	223 (60.3)	161 (51.9)		Reference
C	147 (39.7)	149 (48.1)		0.716 (0.527-0.973)

Values are presented as number (%)

*Evaluated by chi-square test

†Calculated by multivariable logistic regression including body mass index and PARP1 Val762Ala polymorphism

Discussion

In the present study, I evaluated the relationship between the PARP-1 Val762Ala polymorphism and POP. I found that the PARP-1 Val762Ala polymorphism is associated with a decreased risk of POP (Figure 2). To the best of my knowledge, this is the first report to demonstrate an association between the PARP-1 Val762Ala polymorphism and the risk of POP (MEDLINE; 1900-August 2012; English language; search terms: “Pelvic organ prolapse” or “POP” and “Polymorphism” or “Poly(ADP-ribose) polymerase” or “PARP”).

Although the pathophysiology of POP has not been clearly elucidated, growing evidence supports the hypothesis that oxidative stress is one of the contributing factors in the development of POP (9,16-19). Especially, Kim et al (9) revealed that oxidative stress biomarkers and markers for apoptosis were elevated in POP patients. Oxidative stress seems to be an initiator or propagator of apoptotic cell death in the uterosacral ligament of POP patients and a component of the pathologic process underlying progression of the disease (The key results of the experiment are shown in Figure 3-5). Poly(ADP-ribosyl)ation is a post-translational protein modification carried out by the poly(ADP-ribose) polymerase (PARP) enzyme, and plays a crucial role as a sensor and a response mediator in the cellular response to genotoxic stress signals, including oxidative stress (12). Human PARPs comprise a family of 18 enzymes sharing a conserved catalytic domain (20). Among them, PARP-1 is responsible for more than 90% of the cellular poly(ADP-ribosyl)ation capacity (21). PARP-1 consists of three main domains: the DNA-binding domain, the automodification domain, and the catalytic domain (20). The Val762Ala polymorphism, which exchanges base T for base C at codon 762 in exon 17 and results in the substitution of valine by alanine in the

catalytic domain, has been reported to be associated with altered PARP-1 activity, with the Ala allele decreasing the enzymatic activity (13,22).

Given the contribution of PARP-1 to DNA repair and maintenance of genomic integrity, I expected that the Ala allele might be present at higher frequencies in patients with POP because reduced BER for reactive oxygen species-induced DNA damage may trigger downstream apoptotic machinery and consequent cell death. However, the results showed the opposite pattern, instead implying that the Val762Ala polymorphism contributes to the pathogenesis of POP through mechanisms other than defective DNA repair.

In addition to its role in mediating DNA repair, PARP-1 plays an important role in determining cellular outcomes in response to DNA damage, which depend on the type, strength, and duration of genotoxic stimuli (12). In response to low levels of genotoxic stress, PARP-1 promotes cell survival partly through DNA repair, whereas severe or prolonged stress (e.g., oxidative stress) triggers PARP-1 overactivation and the induction of cell death (Figure 6). At least two distinct mechanisms have been proposed to explain PARP-1-induced cell death. First, overactivation of PARP-1 depletes stores of NAD^+ and ATP, causing subsequent energy failure and necrotic cell death (23)(Figure 7). Second, the PAR polymer, the major product of PARP-1 activation, stimulates apoptosis-inducing factor-dependent apoptotic cell death (24,25)(Figure 8). A vast body of experimental studies support the idea that PARP-1-induced cell death plays an important role in tissue injury or organ dysfunction in oxidative stress-related diseases, including ischemia-reperfusion injury (26), localized or systemic inflammation (27), and diabetes (28).

PARP-1 also contributes to oxidative stress-related cellular injury by promoting inflammation beyond safe levels Oxidative stress generates single-

stranded DNA breaks, which promote PARP-1-induced necrotic cell death, leading to the release of cellular contents into the surrounding tissue. Activated PARP-1 potentiates nuclear factor-kappa B (NF-κB) activation with the subsequent up-regulation of NF-κB-dependent pro-inflammatory genes (e.g., inducible nitric oxide synthase, intracellular adhesion molecule-1, tumor necrosis factor-alpha). These processes promote the recruitment of a larger number of activated leukocytes to inflamed sites, thereby increasing oxidative stress and triggering more DNA strand breakage. This cycle is renewed by multiple positive feedback cycles (29)(Figure 9).

Based on these actions of PARP-1, I postulate that the PARP-1 Val762Ala polymorphism might reduce the risk of POP by lowering the likelihood of PARP-1-induced apoptotic/necrotic cell death and undesirable inflammation under oxidative stress. The results appear to support this hypothesis. Moreover, when I performed re-analysis excluding the women with mild POP in order to maximize the genetic effect by evaluating more extreme phenotypes, a stronger association was observed.

One potential limitation of the present study is that it was a hospital-based, case-control study. Therefore, the control group may not represent the general population. However, the allele frequency in the control group was similar to that found in other Korean studies (14, 30-33). In addition, recruiting control subjects from postmenopausal women has the advantage of minimizing the probability that women who may someday be affected by POP are included in the control group. This factor may favorably influence these results. Another limitation of this study is that ethnically homogenous Korean women were included. In the HapMap database, the frequency of the minor C allele in Asians (45.6%) was significantly higher than that of Caucasian (16.7%) or African (0.8%) populations (34). In fact, the association between the PARP-1

polymorphism and cancer risk was different in Asian and Caucasian populations (35). Therefore, these results should be interpreted with caution until validated in larger, population-based studies including different ethnic groups.

In conclusion, the present study provides the first evidence that the PARP-1 Val762Ala polymorphism reduces the risk of POP, implying that this polymorphism may function as a determinant of individual susceptibility to POP.

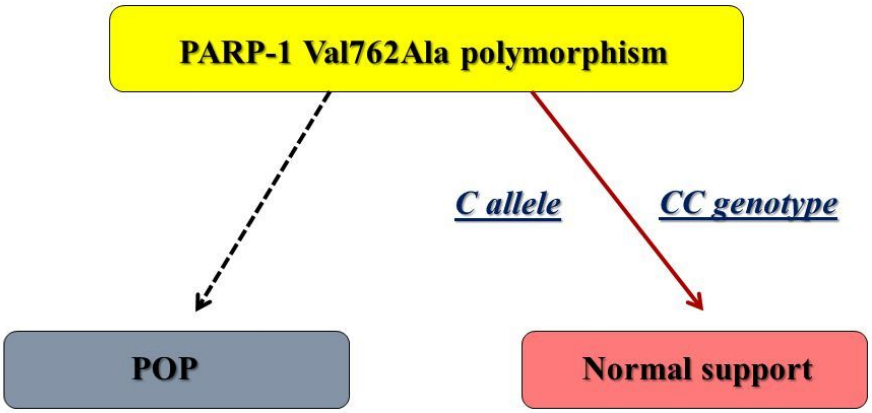

Figure 2. Schematic outline of the results

The subjects who have C allele and CC genotype in PARP-1 Val762Ala polymorphism are prone to be normal support than POP.

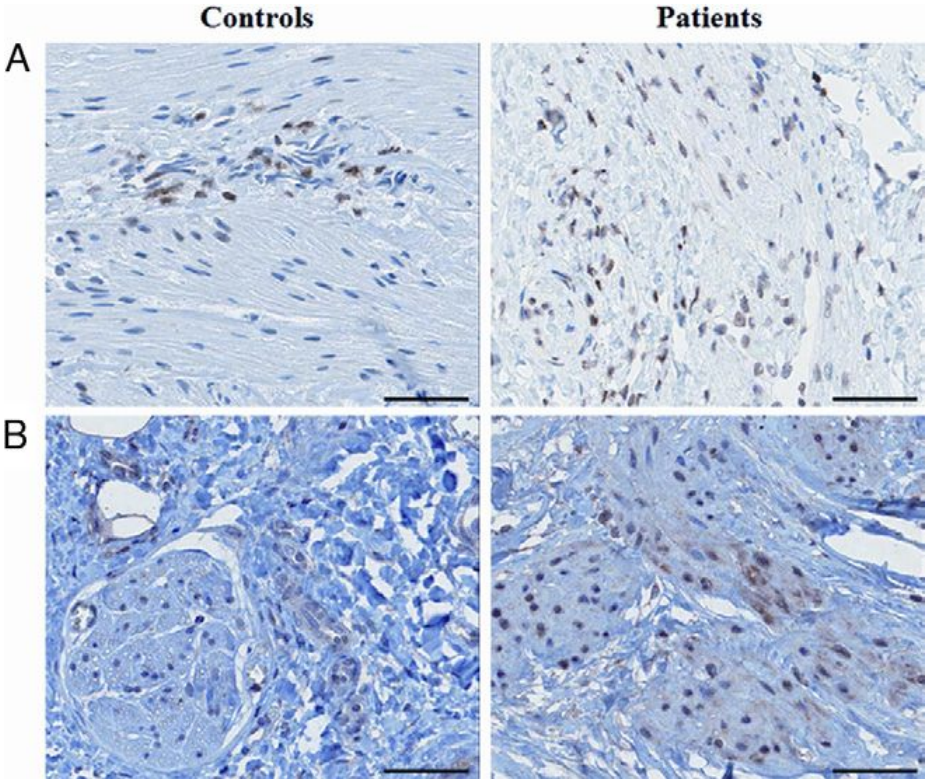

Figure 3. Immunohistochemical staining for 8-OHdG (A) and 4-HNE (B) in uterosacral ligaments (reduced from x400, scale bar=50μm) (36)

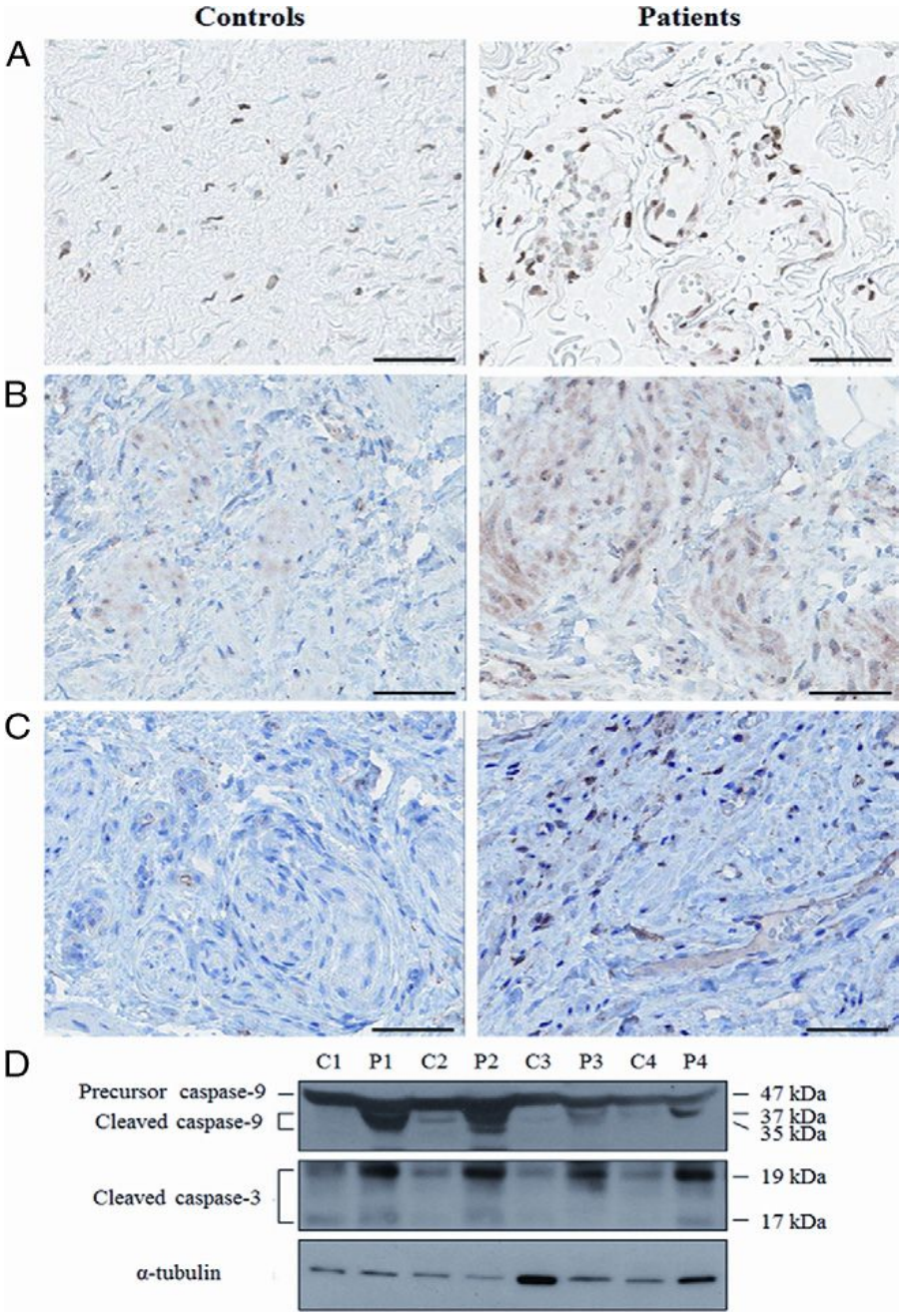

Figure 4. TUNEL assay (A), immunohistochemical staining for cleaved caspase-3 (B) and cytochrome c (C) in USLs (reduced from x400, scale bar=50μm), and expression of cleaved caspase-3 and caspase-9 protein on Western blot analysis (D) (36)

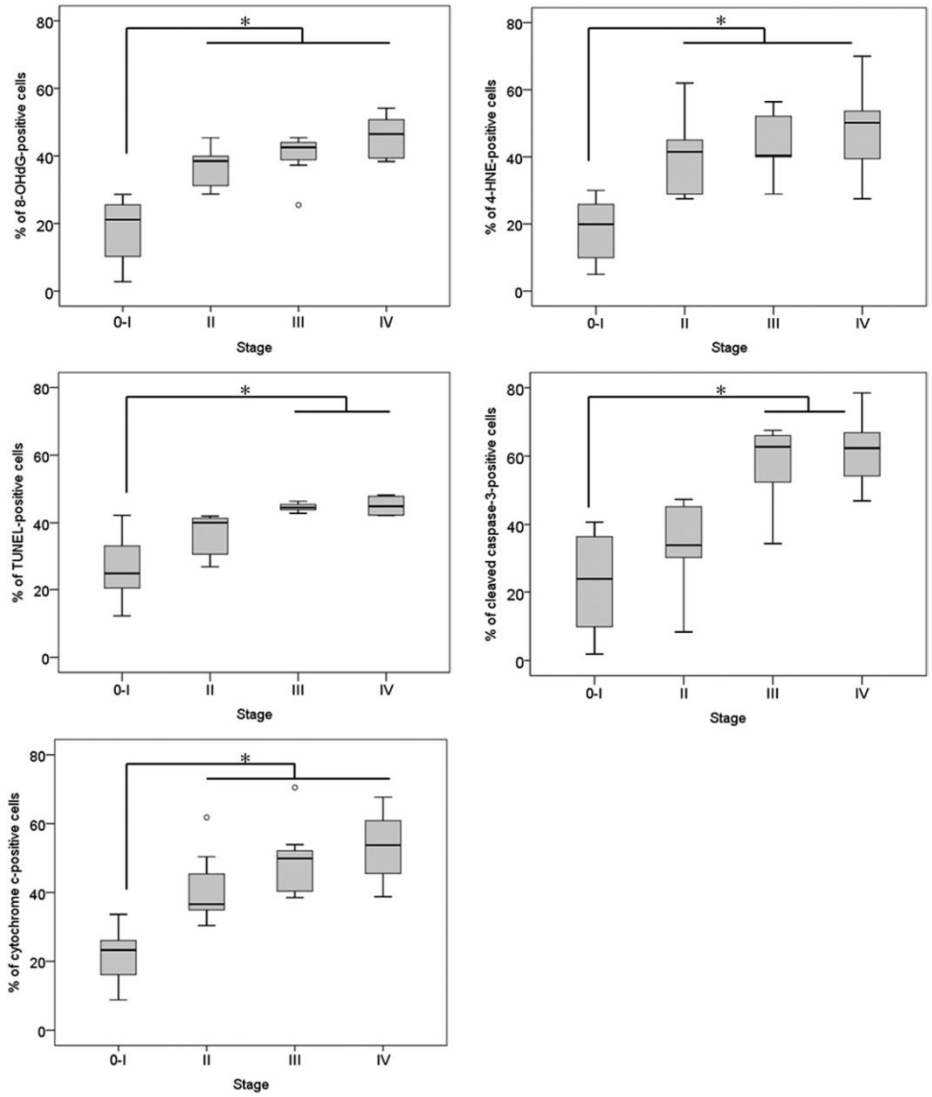

Figure 5. Percentage of cells immunopositive for oxidative stress biomarkers and markers of mitochondrial apoptosis in USLs over different POP-Q stage according to C point (asterisk indicates $p < 0.01$) (36)

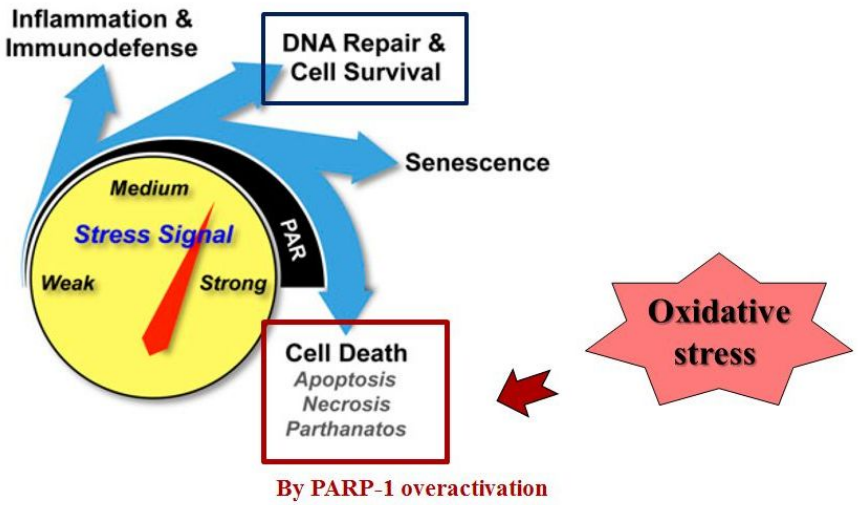

Figure 6. Different actions of PARP-1 according to the type, strength, and duration of genotoxic stimuli (37)

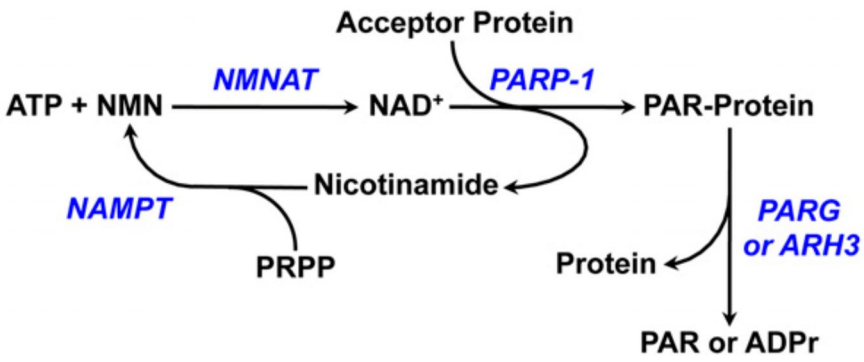

Figure 7. The PARP-1 dependent NAD^+ metabolic cycle (38)

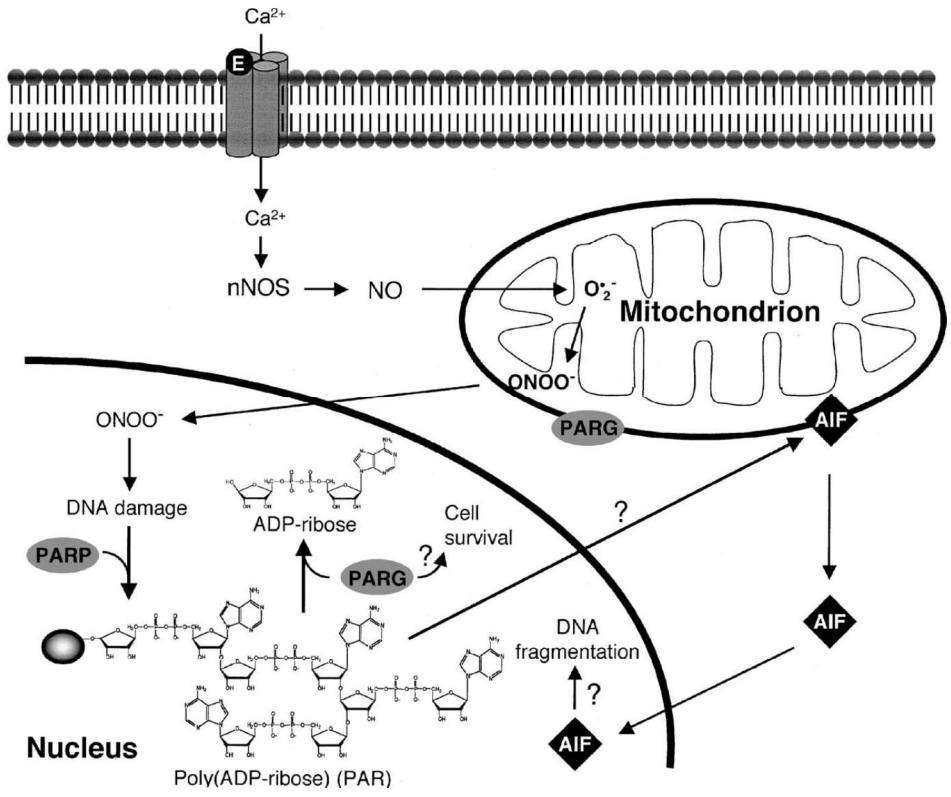

Figure 8. Poly(ADP-ribose) polymerase-1-dependent cell death mediated by apoptosis-inducing factor (39)

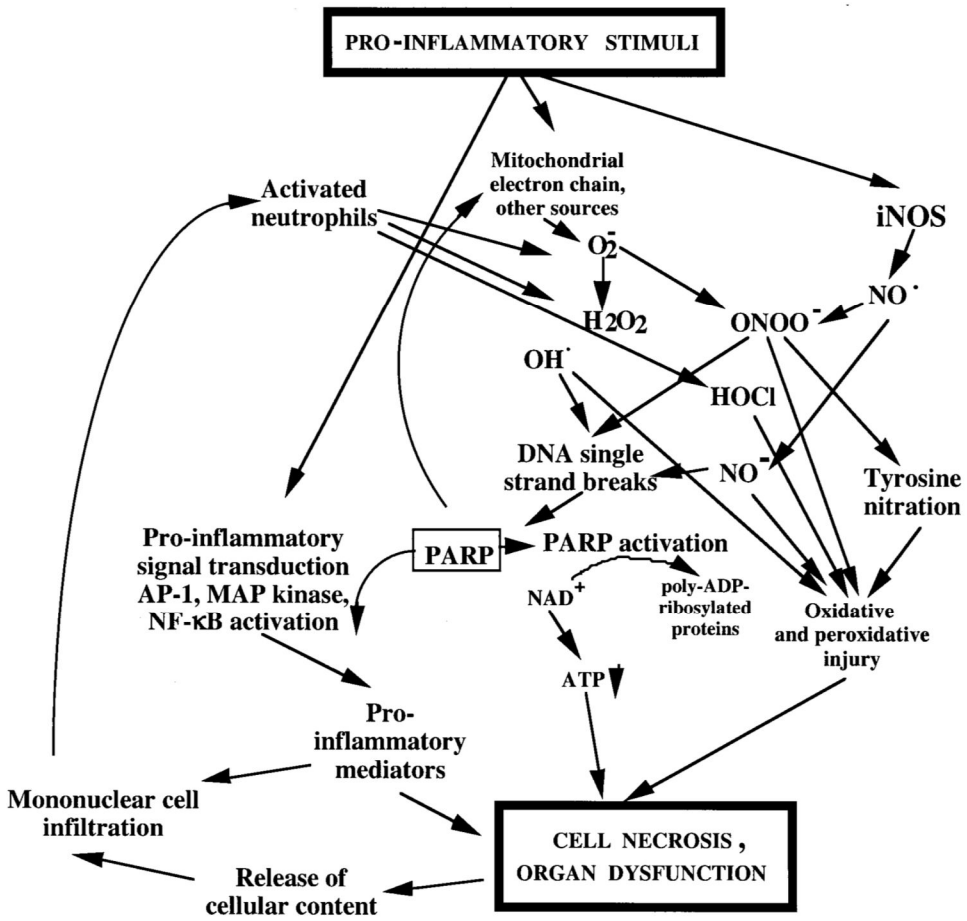

Figure 9. Proposed scheme of PARP-dependent and PARP-independent cytotoxic pathways in local and systemic inflammation (40)

References

1. Jelovsek JE, Barber MD. Women seeking treatment for advanced pelvic organ prolapse have decreased body image and quality of life. Am J Obstet Gynecol 2006;194:1455-61.

2. Subak LL, Waetjen LE, van den Eeden S, Thom DH, Vittinghoff E, Brown JS. Cost of pelvic organ prolapse surgery in the United States. Obstet Gynecol 2001;98:646-51.

3. Wu JM, Hundley AF, Fulton RG, Myers ER. Forecasting the prevalence of pelvic floor disorders in U.S. Women: 2010 to 2050. Obstet Gynecol 2009;114:1278-83.

4. 대한민국 통계청. 장래인구추계 2010-2060 [Internet]. 2011 [updated 2011 Dec 7; cited 2012 Dec 15]. Available from: http://kostat.go.kr/portal/korea/kor_nw/2/1/index.board?bmode=read&bSeq=&aSeq=252623&pageNo=1&rowNum=10&navCount=10&currPg=&sTarget=title&sTxt

5. Jelovsek JE, Maher C, Barber MD. Pelvic organ prolapse. Lancet 2007;369:1027-38.

6. Altman D, Forsman M, Falconer C, Lichtenstein P. Genetic influence on stress urinary incontinence and pelvic organ prolapse. Eur Urol 2008;54:918-22.

7. Takacs P, Gualtieri M, Nassiri M, Candiotti K, Medina CA. Vaginal smooth muscle cell apoptosis is increased in women with pelvic organ prolapse. Int Urogynecol J Pelvic Floor Dysfunct 2008;19:1559-647.

8. Takacs P, Nassiri M, Gualtieri M, Candiotti K, Medina CA. Uterosacral Ligament Smooth Muscle Cell Apoptosis Is Increased in Women With Uterine Prolapse. Reprod Sci 2009;16:447-52.

9. Kim EJ, Chung NH, Park SH, Lee KH, Kim SW, Kim JY, et al.

Involvement of oxidative stress and mitochondrial apoptosis in the pathogenesis of pelvic organ prolapse. J Urol. Forthcoming 2013.

10. Hoeijmakers JH. Genome maintenance mechanisms for preventing cancer. Nature 2001;411:366-74.

11. Oka S, Hsu CP, Sadoshima J. Regulation of cell survival and death by pyridine nucleotides. Circ Res 2012;111:611-27.

12. Luo X, Kraus WL. On PAR with PARP: cellular stress signaling through poly(ADP-ribose) and PARP-1. Genes Dev 2012;26:417-32.

13. Wang XG, Wang ZQ, Tong WM, Shen Y. PARP1 Val762Ala polymorphism reduces enzymatic activity. Biochem Biophys Res Commun 2007;354:122-6.

14. 대한민국 통계청. 장래인구추계 2010-2060 [Internet]. 2011 [updated 2011 Dec 7; cited 2012 Dec 15]. Available from: http://kostat.go.kr/portal/korea/kor_nw/2/1/index.board?bmode=read&bSeq=&aSeq=252623&pageNo=1&rowNum=10&navCount=10&currPg=&sTarget=title&sTxt. Figure 4, 성 및 연령별 인구피라미드, 2010-2060; p.7.

15. Jin XM, Kim HN, Lee IK, Park KS, Kim HJ, Choi JS, et al. PARP-1 Val762Ala polymorphism is associated with reduced risk of non-Hodgkin lymphoma in Korean males. BMC Med Genet 2010;11:38.

16. Jackson SR, Avery NC, Tarlton JF, Eckford SD, Abrams P, Bailey AJ. Changes in metabolism of collagen in genitourinary prolapse. Lancet 1996;347:1658-61.

17. Sun MJ, Cheng WL, Wei YH, Kuo CL, Sun S, Tsai HD, et al. Low copy number and high 4977 deletion of mitochondrial DNA in uterosacral ligaments are associated with pelvic organ prolapse progression. Int Urogynecol J Pelvic Floor Dysfunct 2009;20:867-72.

18. Tseng LH, Chen I, Lin YH, Chen MY, Lo TS, Lee CL. Genome-based

expression profiles study for the pathogenesis of pelvic organ prolapse: an array of 33 genes model. Int Urogynecol J 2010;21:79-84.

19. Choy KW, Liu YM, Chu CY, Wang CC, Lui WT, Lee LL, et al. High isoprostane level in cardinal ligament-derived fibroblasts and urine sample of women with uterine prolapse. BJOG 2008;115:1179-83.

20. Amé JC, Spenlehauer C, de Murcia G. The PARP superfamily. Bioessays 2004;26:882-93.

21. Virág L. Structure and function of poly(ADP-ribose) polymerase-1: role in oxidative stress-related pathologies. Curr Vasc Pharmacol 2005;3:209-14.

22. Lockett KL, Hall MC, Xu J, Zheng SL, Berwick M, Chuang SC, et al. The ADPRT V762A genetic variant contributes to prostate cancer susceptibility and deficient enzyme function. Cancer Res 2004;64:6344-8.

23. Ha HC, Snyder SH. Poly(ADP-ribose) polymerase is a mediator of necrotic cell death by ATP depletion. Proc Natl Acad Sci U S A 1999;96:13978-82.

24. Yu SW, Wang H, Poitras MF, Coombs C, Bowers WJ, Federoff HJ, et al. Mediation of poly(ADP-ribose) polymerase-1-dependent cell death by apoptosis-inducing factor. Science 2002;297:259-63.

25. Yu SW, Andrabi SA, Wang H, Kim NS, Poirier GG, Dawson TM, et al. Apoptosis-inducing factor mediates poly(ADP-ribose) (PAR) polymer-induced cell death. Proc Natl Acad Sci U S A 2006;103:18314-9.

26. Szabó C. Pharmacological inhibition of poly(ADP-ribose) polymerase in cardiovascular disorders: future directions. Curr Vasc Pharmacol 2005;3:301-3.

27. Cuzzocrea S. Shock, inflammation and PARP. Pharmacol Res 2005;52:72-82.

28. Obrosova IG, Julius UA. Role for poly(ADP-ribose) polymerase

activation in diabetic nephropathy, neuropathy and retinopathy. Curr Vasc Pharmacol 2005;3:267-83.

29. Virág L, Szabó C. The therapeutic potential of poly(ADP-ribose) polymerase inhibitors. Pharmacol Rev 2002;54:375-429.

30. Lee KA, Bang SY, Park BL, Kim JH, Shin HD, Bae SC. Lack of association between poly(ADP-ribose) polymerase (PARP) polymorphisms and rheumatoid arthritis in a Korean population. Rheumatol Int 2012;32:91-6.

31. Kim J, Pyun JA, Cho SW, Lee K, Kwack K. Lymph node metastasis of gastric cancer is associated with the interaction between poly (ADP-ribose) polymerase 1 and matrix metallopeptidase 2. DNA Cell Biol 2011;30:1011-7.

32. Hur JW, Sung YK, Shin HD, Park BL, Cheong HS, Bae SC. Poly(ADP-ribose) polymerase (PARP) polymorphisms associated with nephritis and arthritis in systemic lupus erythematosus. Rheumatology (Oxford) 2006;45:711-7.

33. Choi JE, Park SH, Jeon HS, Kim KM, Lee GY, Park RW, et al. No association between haplotypes of three variants (codon 81, 284, and 762) in poly(ADP-ribose) polymerase gene and risk of primary lung cancer. Cancer Epidemiol Biomarkers Prev 2003;12:947-9.

34. National Center for Biotechnology Information (NCBI). The Single Nucleotide Polymorphism database (dbSNP). Available at: http://www.ncbi.nlm.nih.gov/projects/SNP/. Retrieved August 18, 2012.

35. Yu H, Ma H, Yin M, Wei Q. Association between PARP-1 V762A polymorphism and cancer susceptibility: a meta-analysis. Genet Epidemiol 2011;36:56-65.

36. Kim EJ, Chung NH, Park SH, Lee KH, Kim SW, Kim JY, et al. Involvement of oxidative stress and mitochondrial apoptosis in the pathogenesis of pelvic organ prolapse. J Urol. Forthcoming 2013. Figure 1-3;

p. 4-5.

37. Luo X, Kraus WL. On PAR with PARP: cellular stress signaling through poly(ADP-ribose) and PARP-1. Genes Dev 2012;26:417-32. Figure 6, PARP-1 functions as a cellular rheostat. PARP-1 promotes different cellular responses to different types and levels of stress signals. As the strength of stress stimulus increases, the levels of PARP-1 activity and PAR synthesis increase, leading to different cellular outcomes; p. 426.

38. Luo X, Kraus WL. On PAR with PARP: cellular stress signaling through poly(ADP-ribose) and PARP-1. Genes Dev 2012;26:417-32. Figure 2(B), The PARP-1 dependent NAD^+ metabolic cycle; p. 418.

39. Koh DW, Dawson TM, Dawson VL. Mediation of cell death by poly(ADP-ribose) polymerase-1. Pharmacol Res. 2005;52(1):5-14. Figure 2, Poly(ADP-ribose) polymerase-1-dependent cell death mediated by AIF; p. 10.

40. Virág L, Szabó C. The therapeutic potential of poly(ADP-ribose) polymerase inhibitors. Pharmacol Rev 2002;54:375-429. Figure7, Proposed scheme of PARP-dependent and PARP-independent cytotoxic pathways involving nitric oxide ($NO^{\cdot}$), hydroxyl radical ($OH^{\cdot}$), and peroxynitrite ($ONOO^-$) in local and systemic inflammation; p. 393.

국문 초록

서론: 산화스트레스로 인해 유발되는 세포자멸사는 골반장기탈출증의 발생에 영향을 미친다. Poly(ADP-ribose) polymerase-1 (PARP-1)은 산화스트레스에 대한 세포 반응의 주요 매개체이기 때문에, PARP-1 유전자의 다양성은 골반장기탈출증의 병인에 중요한 역할을 할 수 있다. 따라서 본 연구에서 골반장기탈출증과 PARP-1 Val762Ala 유전자 다형성 사이에 연관성이 있는지를 알아보고자 하였다.

방법: 총 370명의 여성이 연구에 포함되었다. 환자군에는 POP-Q 병기 제 2기 이상의 215명의 여성이 포함되었으며, 대조군에는 부인과 검진 목적 또는 양성 부인과 질환으로 방문한 여성들 중 POP-Q 병기 제 0 또는 I기의 정상골반 지지를 가진 155명의 폐경 여성이 포함되었다. 이들로부터 말초혈액을 채취하여 DNA purification kit을 이용하여 genomic DNA를 추출한 후 TaqMan assay를 이용한 실시간 중합효소 연쇄반응법(real-time polymerase chain reaction)을 통해 PARP-1 Val762Ala에 대한 유전자형을 구분하였다.

결과: 유전자형의 분포에 있어서 두 군간에 유의한 차이를 보였으며(TT/TC/CC 33.5%/51.6%/14.9% vs. 29.0%/45.8%/25.2%, p=0.046), 대립유전자 빈도에 있어서도 환자군의 C 대립유전자의 빈도가 대조군에 비해 유의하게 낮았다(40.7% vs. 48.1%, p=0.046). TT 유전자형을 가진 여성들에 비해 CC 유전자형을 가진 여성들의 경우

골반장기탈출증 발생 위험이 0.513배 낮았으며(95% CI, 0.282-0.934; p=0.029), T 대립유전자를 가진 여성들에 비해 C 대립유전자를 가진 여성들의 경우 골반장기탈출증 발생 위험이 0.742배 낮았다(95% CI, 0.553-0.997; p=0.047). 또한 경도의 골반장기탈출증(제 II기)을 제외하고 재분석해 보았을 때, 이러한 연관성은 더 크게 관찰되었다.

결론: 본 연구결과는 PARP-1 Val762Ala 유전자 다형성이 골반장기탈출증 발생 위험을 감소시킨다는 것을 시사한다.

주요어: 골반장기탈출증, 산화스트레스, 단일유전자변이, PARP-1

학 번: 2011-21976