The expression of glutathione peroxidase-1 and the anabolism of collagen regulation pathway transforming growth factor-β_1-connective tissue growth factor in women with uterine prolapse and the clinic significance

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Summary

Objectives: To investigate the expression of the anabolism of collagen regulation pathways connective tissue growth factor (CTGF) - transforming growth factor-beta1 (TGF-β1) and glutathione peroxidase-1 (GPx1) in women with uterine prolapse and a study of the clinic significance. *Materials and Methods:* The expression of TGF-β1, CTGF, and GPx1 was detected by immunohistochemical staining in pubocervical fascia tissue of 30 women with uterine prolapse, including ten cases of POP-QII, ten cases of POP-QIII, ten cases of POP-QIV, and 20 cases were control group with non-prolapse and non-malignant lesions. *Results:* There was a negative correlation between the POP-Q and expression of TGF-β1. With the increase of POP-Q degree, the expression degree of TGF-β1 decreased correspondingly, which also applied to CTGF and GPx1. On the other hand, there was a positive correlation between TGF-β1 and CTGF. The synergistic change trend was found between TGF-β1 and CTGF. It could also be seen between CTGF and GPx1 and betweenTGF-β1 and GPx1. *Conclusion:* The expression of the antioxidase GPx1 in pelvic support structure of POP women was decreased, which resulted in the antioxidation reduced. It could break the balance of oxidation and antioxidation in pelvic support structure, and may induce an increase of ROS level and the down-regulation of TGF-β1-CTGF pathway. It could inhibit the anabolism of collagen and injury the pelvic support structure, thus promoting the occurrence and development of POP.

Key words: Uterine prolapse; TGF-β1; CTGF; GPx1.

Introduction

Uterine prolapse is one of the most common types in pelvic organ prolapse (POP), and its exact etiology is still unknown. The researches showed that the abnormal collagen metabolism in pelvic floor fascia connective tissue was the key of uterine prolapse; matrix metalloproteinases (MMPs) was involved in the occurrence and development of POP through regulating the collagen catabolism [1-6]. Recent researches showed that transforming growth factor-β1 (TGF-β1)-connective tissue growth factor (CTGF) pathway regulated the collagen metabolism; however, the function in uterine prolapse is still unknown [7-10].

Multiple studies have shown that superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) constitute the antioxidation defense system of organisms, the decrease of the antioxygenic enzymes activity up-regulate the oxidative stress in cells, then affect the activity of collagen metabolism enzymes, such as MMPs and tissue inhibitor of metalloproteinases (TIMPs). One study found that the concentration of selenium and GPx was lower in camel with uterine prolapse than in normal camel [11]. Choy *et al.* found that the level of isoprostane increased in the cardinal ligament and urine of patients with POP, which suggested that oxidative stress might be one etiology of POP [12]. In addition, research findings reported that the level of plasma selenium and GPx was lower in camel with uterine prolapse than that in the normal camel.

So, the authors hypothesized that the decrease of GPx activity in pelvic floor fascia tissue would reduce the antioxygen stress ability. It may be the important reason why the oxidative stress increases in pelvic floor tissue and GPx activity was closely related with TGF-β1-CTGF regulating pathway.

Materials and Methods

Materials included: rabbit anti-human GPx1 polyclonal antibody, rabbit anti-human TGF-β1, CTGF polyclonal antibody, horseradish peroxidase labelled goat anti-rabbit polyclonal antibody, DBA, and an SP Kit.

Samples: approximately 100 mg of tissue sample was obtained with a sample intraoperatively from the pubocervical fascia tissue from each patient.

Methods: samples of the cervical fascia tissue were collected from 50 women undergoing vaginal hysterectomy at the present hospital from September 2010 to June 2011. Thirty of the patients with POP studied were placed into Group 1 (n = 10), Group 2 (n = 10), and Group 3 (n = 10), according to Pelvic Organ Prolapse Quantification (POP-Q). POP-Q II is group 1, POP-Q III is group 2, and POP-Q IV is group 3. Twenty cases with other benign gynecological disease were selected as the control group.

Control and prolapse subjects who were smokers or had concomitant malignant pelvic diseases or had been receiving local or systemic hormone replacement therapy, under anti-inflamma-

Revised manuscript accepted for publication December 19, 2012

tory or steroid medications, were excluded from the study. All patients were matched to exclude possible influencing factors such as age, parity, and body mass index. Informed consents were obtained from all participating subjects and the Ethics Committee approval was obtained.

Immunohistochemical staining for GPx1 was performed to determine the presence and distribution of this protein in the pubocervical fascia tissue of POP patients. Semi-quantitative score was used to analyze the staining result. Two investigators who had no idea of the patients' clinical information independently assessed the staining intensity. Preimmune sera was used as a negative control.

The data were analyzed by Chi-square test and Spearman rank correlation analysis. Significance was accepted at $p < 0.05$.

Results

Expression of TGF-β_1, CTGF and GPx1

The positive granules of TGF-β_1 and CTGF appeared dark brown or filemot, which presented a diffuse or focal distribution throughout the cytoplasm (Figures 1A-1D). TGF-β1 expression showed significant decrease, $\chi^2 = 27.242$, $p < 0.05$ (Table 1). CTGF expression also showed significant decrease, $\chi^2 = 23.958$, $p < 0.05$ (Table 2).

The positive granules of GPx1 appeared dark brown or filemot, which presented a focal or diffuse distribution throughout the cytoplasm (Figures 2A-2B). GPx1 expression also showed significant decrease, $\chi^2 = 9.545$, $p < 0.05$ (Table 3).

Correlation between the expression of TGF-β_1 and the POP-Q, CTGF and the POP-Q or GPx1 and the POP-Q

As the ordered category variables, there was a negative correlation between the POP-Q and expressions of TGF-β_1. With the degree of POP-Q increasing,, the expression of TGF-β_1 decreased correspondingly (Table 1). It also could be seen between POP-Q and expression of CTGF (Table 2), and between POP-Q and expression of GPx1 (Table 3).

The correlation analysis between the expression of TGF-β_1 and CTGF, CTGF and GPx1 or TGF-β_1 and GPx1

As the ordered category variables, there was a positive correlation between TGF-β_1 and CTGF. The synergistic change trend was found between TGF-β_1 and CTGF (Table 4) It also could be seen in CTGF and GPx1 (Table 5) and between TGF-β_1 and GPx1 (Table 6).

Discussion

It is generally considered that pregnancy and vaginal childbirth are associated with POP, but the exact etiology is still unknown. Female pelvic tissues were in a complex biomechanical environment with pregnancy, childbirth, high abdominal pressure (chronic cough, constipation, and obesity) etc. In the pathogenesis of POP, some researchers focused on the changes of extracellular matrix components, such as collagen-I, collagen-III, MMP, TIMP, and elastin in connective tissues. So, the decrease of mechanical prop-

Table 1. — *The expression of TGFβ_1 in pubocervical fascia of four groups.*

Groups	Expression of TGFβ1				
	–	+	++	+++	%
POP-QII	30.00 (3/10)	70.00 (7/10)	0	0	70.00 (7/10)
POP-QIII	50.00 (5/10)	50.00 (5/10)	0	0	50.00 (5/10)
POP-QIV	80.00 (8/10)	20.00 (2/10)	0	0	20.00 (2/10)
Total POP	53.33 (16/30)	46.67 (14/30)	0	0	46.67 (14/30)*
Control	10.00 (2/20)	25.00 (5/20)	45.00 (9/20)	20.00 (4/20)	90.00 (18/20)

*The comparison between total POP and control, the $\chi^2 = 27.242$, $p < 0.05$. The correlation coefficient between TGFβ_1 and POP-Q was –0.409, $p < 0.05$.

Table 2. — *Expression of CTGF in pubocervical fascia of four groups.*

Groups	Expression of CTGF				
	–	+	++	+++	%
POP-QII	20.00 (2/10)	80.00 (8/10)	0	0	80.00 (8/10)
POP-QIII	40.00 (4/10)	60.00 (6/10)	0	0	60.00 (6/10)
POP-QIV	90.00 (9/10)	10.00 (1/10)	0	0	10.00 (1/10)
Total POP	50.00 (15/30)	50.00 (15/30)	0	0	50.00 (15/30)*
Control	15.00 (3/20)	25.00 (5/20)	35.00 (7/20)	25.00 (5/20)	85.00 (17/20)

*The comparison between total POP and control, the $\chi^2 = 23.958$, $p < 0.05$. The correlation coefficient between CTGF and POP-Q was –0.572, $p < 0.05$.

Table 3. — *Comparison of expression of GPx1 protein in pubocervical fascia among four groups.*

Groups	Samples (n)	Expression of GPx1 [% (n/n)]				Total positive rate
		–	+	++	+++	
POP-QII	10	40.00 (4/10)	50.00 (5/10)	10.00 (1/10)	0	60.00 (6/10)
POP-QIII	10	80.00 (8/10)	20.00 (2/10)	0	0	20.00 (2/10)
POP-QIV	10	100.00 (10/10)	0	0	0	0
POP Group	30	73.33 (22/30)	23.33 (7/30)	3.33 (1/30)	0	26.67 (8/30)
Control	20	20.00 (4/20)	40.00 (8/20)	30.00 (6/20)	10.00 (2/20)	80.00 (16/20)

* The comparison with the control, $\chi^2 = 9.545$, $p < 0.05$. The correlation coefficient between GPx1 and POP-Q was –0.660, $p < 0.05$.

Table 4. — *Correlation of expression of TGF-β_1 and CTGF in the POP patients.*

TGF-β_1	CTGF				Total
	–	+	++	+++	
–	11	5	0	0	16
+	4	10	0	0	14
++	0	0	0	0	0
+++	0	0	0	0	0
Total	15	15	0	0	30

r = 0.401, $p = 0.028$.

Table 5. — *Correlation of expression of GPx1 and CTGF in the POP patients.*

CTGF	Gpx1				Total
	–	+	++	+++	
–	14	1	0	0	15
+	8	6	1	0	15
++	0	0	0	0	0
+++	0	0	0	0	0
Total	22	7	1	0	30

r = 0.455, $p = 0.012$.

Table 6. — *Correlation of expression of GPx1 and TGFβ1 in the POP patients.*

TGFβ1	Gpx1				Total
	–	+	++	+++	
–	16	0	0	0	16
+	6	7	1	0	14
++	0	0	0	0	0
+++	0	0	0	0	0
Total	22	7	1	0	30

r = 0.641, $p < 0.001$.

Fig. 1A

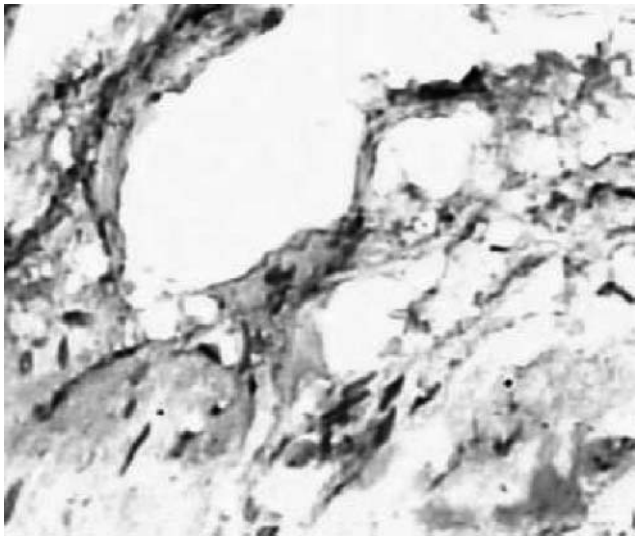


Fig. 1B

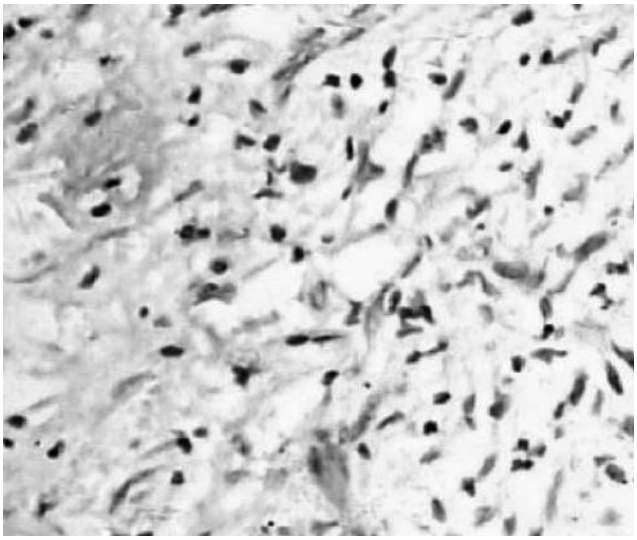


Fig. 1C

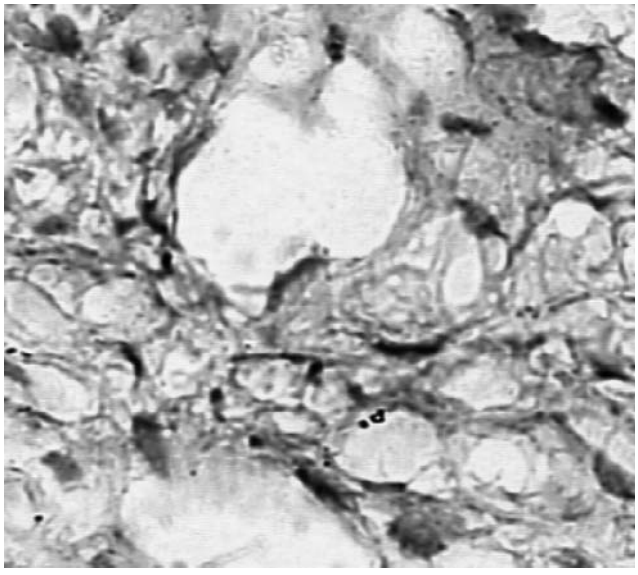


Fig. 1D

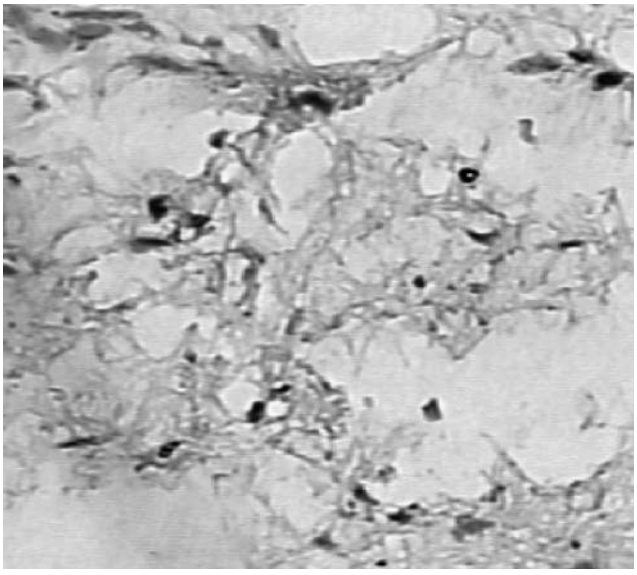


Figure 1. — TGFβ_1 and CTGF expression in pubocervical fascia (two-step staining) (×400).
1A and 1C show the expression of TGFβ_1 in pubocervical fascia in experimental and control group, respectively. 1B and 1D show the expression of CTGF in pubocervical fascia in experimental and control group, respectively.

erty induced by matrix remodeling was the key to the occurrence of POP [13].

Collagen is the main component in ligament and fascia, which determines the toughness of connective tissue. The connective tissue in pelvic floor mainly contained collagen-I and collagen-III [14-17]. The metabolic balance between collagen synthesis and collagen catabolism was broken, which led to pelvic floor tissue becoming weak and lax. It would ultimately result in the occurrence of POP [18, 19].

Under normal physiological conditions, the oxidation-antioxidation system maintains dynamic balance, which not only guarantees the physiological function of the normal oxidative stress reaction, but also prevents the injury of ROS. Only with the ROS overload or insufficient expression of antioxidation enzymes, the dysequilibrium of oxidation-antioxidation system would injure cells and tissues. The oxidation-antioxidation system is the basic of the health.

GPx is an important selenium protein in organism. Selenium is the active center of the enzyme, and its activity can reflect the level of selenium. GPx1 is one of the isozymes, and is widely distributed in the cytoplasms and mitochondria of every tissue cells. The expression of GPx1 reflects the level of selenium in tissue, and also is closely related to the ability of antioxidant. Some studies found that oxidative stress interferes with collagen metabolism in fibroblast cells [20-21].

Other factors such as pregnancy, childbirth, chronic constipation, and chronic cough, which increase intra-abdom-

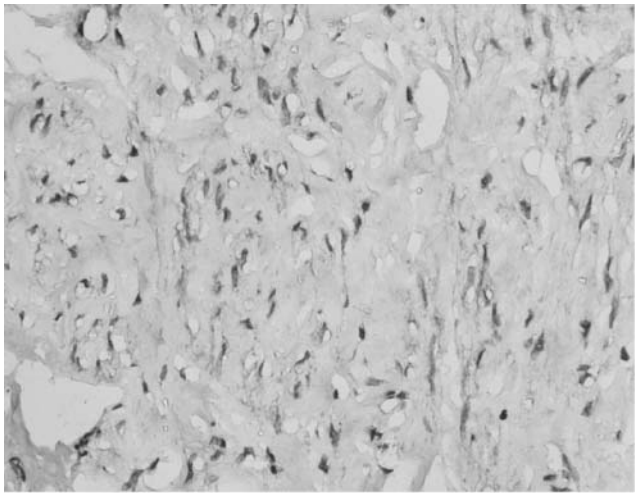
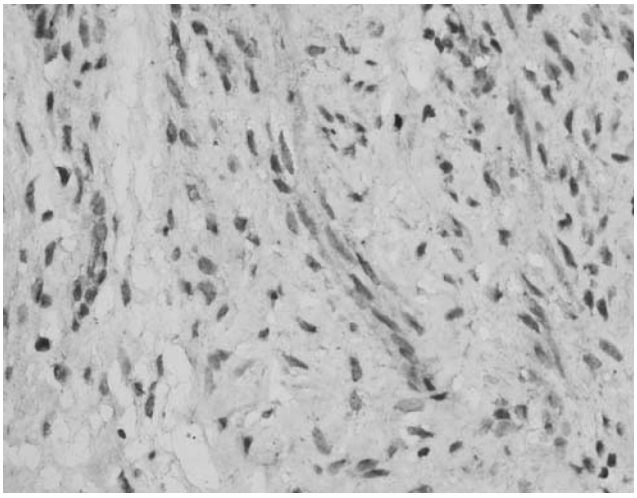
Fig. 2A   Fig. 2B

Figure 2. — GPx1 expression in pubocervical fascia (×400).
2A and 2B show the expression of GPx1 in pubocervical fascia in experimental and control group, respectively.

inal pressure, also are the important causes of POP. Dan found that mechanical strain changes the fibroblast cell morphology in uterosacral ligament and regulates the expression of collagen-I, collagen-III, and MMP-1 [22]. Excessive mechanical strain increases the level of ROS in cells, and then up-regulates the activity of MMPs to fasten the degradation of collagen [23-25]. Therefore, pregnancy, childbirth, chronic constipation, and chronic cough may result in the occurrence and development of POP by inducing the oxidative stress.

In this study, it was first found that the expression of GPx1, an antioxidase, decreased significantly in the pelvic floor fascia tissue of patients with POP, which negatively correlated with the degree of POP-Q. This study suggests that the increasing of mechanical strain or the decreasing expression of GPx1 could break the oxidation-antioxidation system balance of fibroblast cells in pelvic floor supporting tissue, and up-regulate ROS to disturb the metabolic balance of collagen synthesis, which was the key to the occurrence of POP.

In addition, the authors also found that the TGF-β_1-CTGF regulating pathway was decreased in the pelvic floor fascia tissue of patients with POP, and negatively correlated with the degree of POP-Q and positively correlated with GPx1. It could confirm that the expression of GPx1 decreased, which would make the antioxidation weak, increase ROS level in cells, down-regulate TGF-β_1-CTGF pathway, and inhibit the collagen synthesis. The increase of ROS in cells would up-regulate the activity of enzymes such as MMPs and fasten the collagen decomposition.

Conclusion

The expression of the antioxidase GPx1 in pelvic support structure of POP women decreased, which resulted in the antioxidation reduced. It could break the balance of oxidation and antioxidation in pelvic support structure, and may induce the increase of ROS level and the down-regulation of TGF-β_1-CTGF pathway. It could inhibit the anabolism of collagen and injure the pelvic support structure, thus promoting the occurrence and development of POP. In conclusion, the authors provide the hypothesis that the mechanism of POP may be the oxidation-antioxidation system disequilibrium. So, how to regulate the balance is the key to prevent and cure POP.

Acknowledgment

The authors are grateful to the National Nature Science Foundation of China (Project no. 81270684) for its financial support.

References

[1] Moalli P.A., Shand S.H., Zyczynski H.M., Gordy S.C., Meyn L.A.: "Remodeling of Vaginal connective tissue in patients with prolapse". *Obstet. Gynecol*., 2005, *106,* 953.
[2] Curry T.E. Jr., Osteen K.G.: "The matrix metalloproteinase system changes regulation and impact throughout the ovarian and uterine reproductive cycle". *Endocr. Rev.*, 2003, *24,* 428.
[3] Chen B., Wen Y., Wang H., Polan M.L.: "Differences in estrogen modulation of tissue inhibitor of matrix metalloproteinase-l and matrix metalloproteinase-l expression in cultured fibroblasts from continent and incontinent women". *Am. J. Obstet. Gynecol.,* 2003, *189*, 59.
[4] Cleutjens J.P., Kandala J.C., Guarda E., Guntaka R.V., Weber K.T.: "Regulation of collagen degradation in the rat myocardium after infarction". *J. Mol. Cell. Cardiol.*, 1995, *27*, 1281.
[5] Eghbali M., Blumenfeld O.O., Seifter S., Buttrick P.M., Leinwand L.A., Robinson T.F. *et al.*: "Localization of types I, III and IV collagen mRNAs in rat heart cells by in situ hybridization". *J. Mol. Cell. Cardiol.,* 1989, *21,* 103.
[6] Eghbali M., Czaja M.J., Zeydel M., Weiner F.R., Zern M.A., Seifter S., Blumenfeld O.O.: "Collagen chain mRNAs in isolated heart cells from young and adult rats". *J. Mol. Cell. Cardiol.,* 1988, *20,* 267.
[7] Klein M.B., Pham H., Yalamanchi N., Chang J.: "Flexor tendon wound healing in vitro : the effect of lactateon tendon cell proliferation and collagen production". *J.H. Surg. Am*., 2001, *26,* 847.

[8] Massague J.: "TGF beta signal transduction". *Annu. Rev. Biochem.,* 1998, *67,* 753.
[9] Chen B., Wen Y., Zhang Z., Wang H., Warrington J.A., Polan M.L.: "Menstrual phase dependent gene expression differences in peri-urethra vagina l tissue from women with stress incontinence". *Am. J. Obstet. Gynecol.,* 2003, *l89,* 89.
[10] Suzme R., Yalcin O., Gurdol F., Gungor F., Bilir A.: "Connective tissue alterations in women with pelvic organ prolapse and urinar y incontinence". *Acta Obstet. Gynecol. Scand.*, 2007, *86,* 882.
[11] Gutierrez C., Corbera J.A., Morales I., Morales M., Navarro R.: "Uterine prolapse in 2 dromedary camels". *Can Vet. J.*, 2001, *42,* 803.
[12] Choy K.W., Liu Y.M., Chu C.Y., Wang C.C., Lui W.T., Lee L.L. *et al*.: "High isoprostane level in cardinal ligament-derived fibroblasts and urine sample of women with uterine prolapse". *BJOG,* 2008, *15*, 1179.
[13] Kerkhof M.H., Hendriks L., Brölmann H.A.: "Changes in connective tissue in patients with pelvic organ prolapse-a review of the current literature". *Int. Urogynecol. J. Pelvic Floor Dysfunct.,* 2009, *20,* 461.
[14] Eriksen H.A., Pajala A., Leppilahti J., Risteli J.: "Increased content of type III collagen at the rupture site of human Achilles tendon". *J. Orthop. Res.,* 2002, *20,* 1352.
[15] Zheng H., Si Z., Kasperk R., Bhardwaj R.S., Schumpelick V., Klinge U. *et al*.: "Recurrent inguinal hernia: disease of the collagen matrix?". *World J. Surg.,* 2002, *26,* 401.
[16] Ewies A.A., Al-Azzawi F., Thompson J.: "Changes in extracellular matrix proteins in the cardinal ligaments of post-menopausal women with or without prolapse: a computerized immunohistomorphometric analysis". *Hum. Reprod.*, 2003, *18*, 2189.
[17] Curry T.E. Jr., Osteen K.G.: "The matrix metalloproteinase system changes regulation and impact throughout the ovarian and uterine reproductive cycle". *Endocr. Rev.,* 2003, *24,* 428.
[18] Moalli P.A., Shand S.H., Zyczynski H.M., Gordy S.C., Meyn L.A.: "Remodeling of vaginal connective tissue in patients with prolapse". *Obstet. Gynecol.*, 2005, *106*, 953.
[19] Moalli P.A., Talarico L.C., Sung V.W., Klingensmith W.L., Shand S.H., Meyn L.A. *et al.*: "Impact of menopause on collagen subtypes in the arcus tendineous fasciae pelvis". *Hum. Reprod.,* 2003, *18*, 2189.
[20] Makpol S., Azura Jam F., Anum Mohd Yusof Y., Zurinah Wan Ngah W.: "Modulation of collagen synthesis and its gene expression in human skin fibroblasts by tocotrienol-rich fraction". *Arch. Med. Sci.,* 2011, *7,* 889.
[21] Siwik D.A., Pagano P.J., Colucci W.S.: "Oxidative stress regulates collagen synthesis and matrix metalloproteinase activity in cardiac fibroblasts". *Am. J. Physiol. Cell. Physiol.,* 2001, *28*, C53.
[22] Shan S.Z., Shi B., Li K.J.: "Regulation of morphology and synthesis function by mechanical stretch in human uterosacral ligament fibroblasts". *Chin. J. Pract. Gynecol. Obstet.,* 2012, *28,* 197.
[23] Cheng W., Li B., Kajstura J., Li P., Wolin M.S., Sonnenblick E.H. *et al.*: "Stretch-induced programmed myocyte cell death". *J. Clin. Invest.,* 1995, *96,* 2247.
[24] Lu D., Kassab G.S.: "Role of shear stress and stretch in vascular mechanobiology". *J.R. Soc. Interface,* 2011, *8,* 1379.
[25] Grote K., Flach I., Luchtefeld M., Akin E., Holland S.M., Drexler H. *et al.*: "Mechanical stretch enhances mRNA expression and proenzyme release of matrix metalloproteinase-2 (MMP-2) via NAD(P)H oxidase-derived reactive oxygen species. Circ Res, 2003, 92 (11): e80.

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