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Stage III-IV uterine prolapse risk factors: sacrouterine ligaments high estrogen receptor alpha and collagen III expression and low elastin expression

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ABSTRACT

Background: Uterine prolapse is common, non-life-threatening, but has a negative impact on women psychosocial and economic life. Damage to levator ani muscle is the early onset of uterine prolapse, while the damage of sacrouterine ligaments aggravates the stage. The strength of sacrouterine ligament depends on tissue cellularity, the formation of collagen I/III ratio, and the decreased expression of elastin. The lower the ratio of collagen I/III, the higher the risk of stage III-IV uterine prolapse. The ratio of collagen I/III formation is allegedly influencing through the expression of estrogen receptor alpha, by increasing collagen III synthesis and decreasing the degradation.

Objective: We aimed to investigate whether high estrogen receptor alpha and collagen III expression, and the low elastin expression in the sacrouterine ligaments were stage III-IV uterine prolapse risk factors.

Method: In March to August 2014, a non-matching case control study was conducted in 3 hospitals in Denpasar, and the materials were processed in the Faculty of Veterinary Medicine Laboratory of Udayana University. The case was uterine prolapse stage III-IV, the control was the non-uterine prolapse. We collected 1.5 cm residual sacrouterine ligaments from the edge of the cervix fixed with 10% buffered formalin from patients who underwent a total hysterectomy. They were examined immunohistochemically to identify estrogen receptor alpha expression, collagen III, and elastin.

Results: Our sample was 44, divided equally between the case and control group. Compared to the control, in the case group, the proportion was significantly higher for the high estrogen receptor alpha expression (OR=5.71, 95%CI 1.56-20.93, p=0.007), high collagen III (OR=6.50, 95% CI 1.64- 25.76, p=0.005), and low elastin (OR=5.40, 95%CI 1.37-21.26, p=0.012).

Conclusion: the high expression of estrogen receptor alpha and collagen III and low expressions of elastin in sacrouterine ligaments served as stage III-IV uterine prolapse risk factors.

Keywords: expression of estrogen receptor alpha, collagen III, elastin, stage III-IV uterine prolapse.

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INTRODUCTION

Uterine prolapse is a common health problem that relates to decreasing life quality, and the number and the severity is increasing along with increasing life expectancy. It is not life-threatening, but has a negative impact on psychosocial and economic life and sexual function. Caused by the weakening of pelvic organ support structure, it is the fall of uterine to the vagina, and the uterus can even be expelled out of the vagina.

The incident was increasing along with increasing age. A study reported a 10% increasing risk of pelvic organ prolapse in every decade addition of the patient age, and the incident was between 43-76%.[1] About 41% happened in 50-79 years old: 34% cystocele, 19% rectocele, and 14% uterine prolapse.[2] A study predicted more than 50% of women with vaginal delivery had any stage of prolapse, and 10-20% had pelvic organ prolapse symptoms.[3] In the US, pelvic organ prolapse operation was 2.7-3.3 in 1.000, and in England, 2 in 1.000 women annually.[4] In Indonesia, the Obstetric and Gynecologic Department of Hasan Sadikin Hospital, Bandung, annual report showed the incident of uterine prolapse in 2007 was 30 cases of stage III-IV, while in Sanglah Hospital, Denpasar, an average of 20 cases of stage III-IV uterine prolapse was operated each year.

The exact cause of stage III-IV uterine prolapse is still unknown. A study mentioned the influences of physiologic and traumatic incident.[5] Another study categorized the risk factors into intrinsic: genetic, race, collagen changes; and extrinsic factors: pregnancy, labor, history of hysterectomy, hormone replacement therapy, menopause and type of works. Vaginal delivery was a dominant risk factor. Direct trauma of labor induces damage and weakened levator ani muscle. If the muscle repair goes normal, the genital hiatus becomes narrowed, the uterine and proximal vagina stays in the normal position, and the sacrouterine ligaments (the uterine hanger) stays in a relaxed position. In the opposite, if the repair fails, the genital hiatus dilates,

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and to maintain the uterine normal position, the sacrouterine ligaments becomes strained for taking over the levator ani main function. A continuous strain accompanied by other risk factors (age, parity, obesity, and type of work) lowers the sacrouterine ligaments strength. The strength decrease depends on metabolic molecular changes in the main structure of sacrouterine ligaments.[6]

The main structure of sacrouterine ligaments consists of cells and extracellular matrix such as collagen, elastin, proteoglycan and glycoprotein. Collagen, the main component, constitutes 70% of the extracellular matrix.[1,7] Metabolic molecular changes are allegedly under the influence of collagen I/III formation ratio and decreasing tissue cellularity.[8] Higher collagen I/III ratio indicates a stronger ligament and a low risk of prolapse, and vice versa.[7,9] Following a successful repair, the sacrouterine ligaments strength cannot completely recover. Thus, uterine prolapse still happens in the lowest stage (I-II). If the repair fails, the prolapse stage is higher (III-IV). Therefore, sacrouterine ligaments have an important role in the stage III-IV uterine prolapse pathogenesis.[10,11]

A decrease in the sacrouterine ligament strength is caused by aging process and loosing neuroendocrine signaling in pelvic tissues. Uterine prolapse higher incident in post menopause proves that hypo estrogenic is an important predisposing factor.[12] The formation of collagen I/III ratio is allegedly influenced by estrogen receptor alpha in the nucleus or membrane cells of fibroblast. The receptor plays role in increasing collagen III synthesis and decreasing the degradation, without having much influence in collagen I metabolism. Thus, collagen I/III ratio decreases. Another component influencing the ligament strength is elastin. Elastin, together with collagen, strengthens and gives elasticity to the tissue. Elastin has a rubber-like character (elastic), easy to stretch and may return to the normal length.[13] In a healing process, a scar formation can extend the ligament tissues and decrease the elasticity and strength. Moreover, if the expression of elastin is low, the strength and elasticity of sacrouterine ligaments decreases.[1]

Molecular mechanism in collagen and elastin metabolism in many situations is still unknown. Collagen and elastin metabolism disturbance can cause loss of tissue strength and elasticity. Several molecular biology studies already done to explain mechanism of stage III-IV uterine prolapse, but the main molecular mechanism underlying the disturbance has not been known.[11,14]

Not all women with the same risk factors has stage III-IV uterine prolapse. Results from previous studies, comparing between the prolapse and the non-prolapse, were still inconsistent about the expression of estrogen receptor alpha, collagen III and elastin in sacrouterine ligaments or in other organs. Kokchi et al acquired collagen I and III expression in vaginal fascia tissue, cardinal ligaments and sacrouterine ligaments higher in uterine prolapse.[15] While Liapis et al. obtained collagen III expression in paravaginal fascia and sacrouterine ligaments lower in uterine prolapse.[16] Gabriel et al. obtained a higher collagen III expression in sacrouterine ligaments in the prolapse cases.[17] Goepel concluded a lower elastin and a higher tenascin expression in uterine prolapse.[18] The variation of extracellular matrix quality and quantity in collagen had already been investigated, but the results were inconsistent and not yet able to explain the main mechanism.[19] By the time this study was conducted, there was no readily available data from any study about the expression of estrogen receptors alpha, collagen III and elastin in sacrouterine ligaments relation with stage III-IV uterine prolapse.

Estrogen receptor alpha has an important role in collagen metabolism that controlling the high and low collagen I/III ratio. The main molecular mechanism causes lower collagen I/III ratio is still unknown. This study aimed to prove the higher estrogen receptor alpha and collagen III expression, with lower elastin expression in sacrouterine ligaments as risk factors in stage III-IV uterine prolapse.

METHODS

This study is a case control study. The study was done at Obstetric and Gynecologic Department of Sanglah Central Public Hospital, Badung Regency Public Hospital, Balimed Hospital. The case was stage III-IV uterine prolapse patients who had a total hysterectomy. The control was non prolapse patients who had a total hysterectomy caused by benign indications such as uterine myoma or abnormal bleeding. The inclusion criteria for both groups was willingness to join the study after an informed consent. The exclusion criteria were malignancy, endometriosis and *elongatio colli*. This study was carried out from March until August 2014. The specimens were sent to the Faculty of Veterinary Medicine Laboratory of Udayana University Denpasar Bali. The specimen was 1.5 cm long taken from the sacrouterine ligament still adhered to the uterine by cutting the ligament with scalpel from uterine cervix margin. Then, it was paraffin blocked and made as an immunohistochemistry preparation.

Immunohistochemistry examination was done to rate the estrogen receptor alpha, the collagen III and the elastin expression. The independent variables were: estrogen receptor alpha expression, collagen III expression, elastin expression in sacrouterine ligaments. The dependent variable was stage III-IV uterine prolapse. The controlled variables were age, parity, body mass index (BMI), type of work.

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Data processing was done with SPSS 16.0 for windows.

RESULTS

We collected a total of 22 patients for each group. Age, parity, and BMI were tested for its distribution normality with Shapiro-Wilk test (p>0.05), see Table 1.

The variables were also tested for homogeneity with Levene's test (p>0.05), see Table 2. An independent T-test was done on age, parity, and BMI, while chi-square was done on job variable. Table 3 showed there was no difference between two groups based on age, parity, and BMI (p>0.05). Based on the workload, 8 (36.36%) had a heavy workload in the case group, and 12 (54.54%) in the control. The chi-square test showed no workload difference (p>0.05).

Table 4 showed that estrogen receptor alpha expression was associated with stage III-IV uterine prolapse (OR 5.71, 95% CI 1.56-20.93, p<0.05). High collagen III expression was also associated with with stage III-IV uterine prolapse (OR 6.50, 95% CI 1.64-25.76, p<0.05). And, low elastin

Table 1 Shapiro-Wilk Test Results

Variable	Subject groups	n	p
Age	case	22	0.209
	control	22	0.094
Parity	case	22	0.065
	control	22	0.121
BMI	case	22	0.768
	control	22	0.724

Table 2 The Homogeneity between Groups

Variable	F	p
Age	2.602	0.114
Parity	0.283	0.598
BMI	0.025	0.876

Table 3 Age, Parity, and BMI Independent T-Test

	Case		Control		
Variable	Mean	SD	Mean	SD	p
Age (year)	51.2	4.66	49.23	2.96	0.08
Parity	3.14	1.08	2.77	0.92	0.24
BMI (kg/m2)	21.89	1.93	23	2.14	0.08

Table 4. Chi-square Test on Estrogen Receptor Alpha, Collagen III, and Elastin Expression

		Groups				
Variable		Case	Control	OR	95% CI	p
Estrogen Receptor Alpha Expression	High	15	6	5.71	1.56-20.93	0.01
	Low	7	16			
Collagen III Expression	High	13	4	6.5	1.64-25.76	0.01
	Low	9	18			
Elastin Expression	Low	12	4	5.4	1.37-21.26	0.01
	High	10	18			

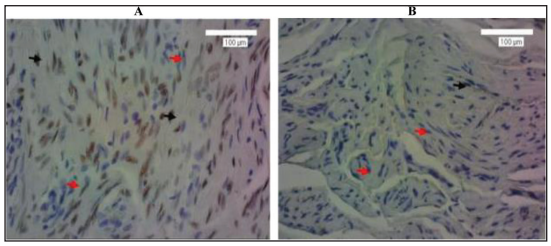

Figure 1 Microscopic Image of Estrogen Receptor Alpha on Sacrouterine Ligaments with Immunohistochemical Stain (A) Case Group, showed an increase in estrogen receptor alpha expression (brown). Black arrows pointed to fibroblasts expressing estrogen receptor alpha. Red arrows showed fibroblasts unexpressing estrogen receptor alpha. (B) Control Group, showed estrogen receptor alpha expression (brown) decreased compared to case. Black arrows pointed to fibroblasts expressing estrogen receptor alpha. Red arrows showed fibroblasts unexpressing estrogen receptor alpha.

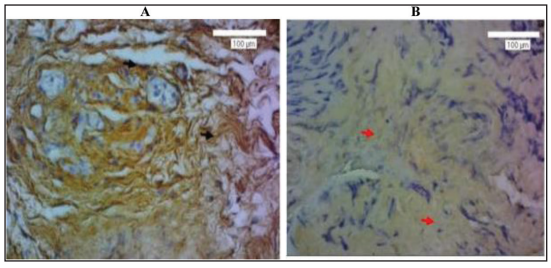

Figure 2 Microscopic Image of Collagen III Expression on Sacrouterine Ligaments with Immunohistochemical Stain (A) Case Group, showed an increase in collagen expression (brown). Black arrow pointed to high intensity collagen expression. (B) Control group, showed less collagen expression (brown) in quality and quantity compared to case. Red arrows pointed to low intensity collagen expression.

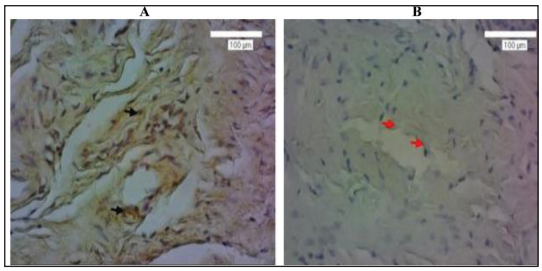

Figure 3 Microscopic Image of Elastin Expression on Sacrouterine Ligaments with Immunohistochemical Stain (A) Control Group, showed an increase in elastin expression (brown). Black arrow pointed high intensity elastin expression. (B) Case Group, showed less elastin expression (brown) in quality and quantity compared to control. Red arrows showed low intensity elastin expression.

expression was associated with stage III- IV uterine prolapse (OR 5.40, 95% CI 1.37-21.26; p<0.05).

DISCUSSION

Age mean in case group was 51.32±4.66 years old and 49.23±2.96 in the control group, p>0.05. Uterine prolapse incidence increases along with age, because of the decrease of muscle tone, especially in the 50s and older.[1,5,20] The effect of age on stage III-IV uterine prolapse incidence is related to hormonal status, especially at menopause period, when the estrogen markedly decreases.[13,21] Low estrogens in a long period increases the collagen III synthesis and decreases the degradation because estrogen maintains the collagenous content (especially type I and III) in connective tissue.[22]

Parity mean in case group was 3.14±1.08 and was 2.77±0.92 in the control, p>0.05. Vaginal delivery is said to be a major risk factor of stage III- IV uterine prolapse.[3,23] The risk of uterine prolapse incidence in women who had vaginal delivery was 8.4 times higher compared to women who never had one. The risk increased 10.9 times if they had more than 4 vaginal deliveries.[2,3,24] In our opinion, parity is related to repeated trauma to levator ani muscles, and it performs only as uterine prolapse precipitating factor. Therefore, multiparous women may not experience a uterine prolapse, and women with low parity or even nulliparous may have a uterine prolapse.

BMI of the case group was 21.89±1.93 and 23.00±2.14 in the control group, p>0.05. Body mass index is proposed as a risk factor of uterine prolapse, a higher BMI causing a higher intraabdominal pressure. Obese women (BMI>30) have 2.5 times greater risk to develop a uterine prolapse compared to women with normal BMI (<23.9). Overall, the case and control group were homogenous based on age, parity, and BMI.

High Estrogen Receptor Alpha Expression

Low estrogen level is related to the increase of stage III-IV uterine prolapse incidence, so that estrogen deficiency has been identified as a risk factor. It underlay the emerging rationale of hormone replacement therapy for stage I-II uterine prolapse. Even though the therapy had been said to give a protection effect and a correction to stage III-IV uterine prolapse, the response on women with stage III-IV uterine prolapse was still uncertain. A study found that serum estradiol concentration and estrogen receptor alpha were lower in pre-menopausal women with stage III-IV uterine prolapse, but no difference in serum estradiol concentration and estrogen receptor alpha in post-menopausal women with the same degree of prolapse. A positive relationship was found between estrogen receptor concentration and the length of menopause, while the estrogen receptor alpha expression and estrogen hormone level in post-menopausal women relationship was inverse.[25]

Copas found estrogen receptor expression in levator ani muscle was increased in women with stage III-IV uterine prolapse, but decreased in those who had been given hormone replacement therapy. This indicated that there an increase in estrogen receptor in stage III-IV uterine prolapse cases was present and the expression was inversely related with estrogen hormonal level.[26]

A study found patients with stress urinary incontinence who had been given estradiol therapy had an increase in immature cross-link collagen concentration. A decrease of total collagen concentration due to estrogen hormone therapy, was said to be precipitated by an increase of matrix metalloproteinase activity (MMP) degrading collagen.[27] Another study showed that estradiol supplementation increased the activity of MMP-2.[27] Furthermore, Moali et al. found an increase of MMP-9 activity in pre- and post-menopausal women with stage III-IV uterine prolapse who had been given hormone replacement therapy.[28] In premenopausal state, estrogen level will be higher compared to postmenopausal state. Furthermore, the longer the duration of menopause, the higher the estrogen receptor alpha expression. Estrogen was said to have a role in decreasing tissue inhibitor of metalloproteinase (TIMP), a substrate which is functioned to inhibit the activity of metalloproteinase (MMP).[1] The end result of increased activity of MMP-2 and MMP-9 activity, along with a decrease activity of TIMP, is the degradation of collagen.

Estrogen and its receptor also influence fibroblast proliferation. Liu et al. found that estradiol hormone therapy in women with stage III-IV uterine prolapse decreased fibroblast proliferation

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in cardinal ligaments.[21] Ewies et al. found hormone replacement therapy decreased estrogen receptor alpha as much as 1.5 to 2 times in uterine prolapse.[13] Liu et al. proposed that hormone replacement therapy decreased the strength of cardinal ligaments due to fibroblasts declining number, along with the inhibition of its proliferation.[21] Bai et al. found estradiol therapy decreased fibroblast proliferation through inhibition of protein p53 and p21, the cell division cycle regulators.[27] The inhibition caused fibroblast cells to keep entering G1 phase in cell division cycle and dividing, even though the concentration has reached saturation level. Actively proliferating fibroblast cannot excrete extracellular matrix, including collagen and elastin. This matrix can only be produced by a resting fibroblast (quiescent state/G0).[28] Hence, the end result of an increasing fibroblast proliferation is the declining collagen and another extracellular matrix. Thus, it increases the risk of stage III-IV uterine prolapse. Our study found estrogen receptor alpha expression was a risk factor for stage III-IV uterine prolapse 5.5 times greater (OR 5.71, 95% CI 1.56-20.93, p<0.05).[29] Our finding supports Copas et al. study which found an increase of estrogen receptor alpha expression in levator ani fascia of uterine prolapse women without hormone replacement therapy.[26]

High Collagen III Expression

Various studies tried to determine the relationship between collagen and stage III-IV uterine prolapse, but the result were varied. Some research found decreased total collagen concentration in premenopausal women with stage III-IV uterine prolapse compared to control, but the collagen I/III ratio was not significantly different.[30,31] Some studies found an increased collagen type III in stage III-IV uterine prolapse at premenopausal or postmenopausal state.[7,13,17,18,28] Another research found a decreased collagen type III in women with stage III-IV uterine prolapse.[16,32] We found high collagen III expression was a risk factor of stage III-IV uterine prolapse (OR 6.50, 95% CI 1.64-25.76, p<0.05) compared to low collagen III expression. We also found an increase in collagen III expression in sacrouterine ligaments in case group compared to control.

It was proposed that an increase of activity and metabolism (turn over) of fibroblasts causes an increase of collagen type III synthesis. An increase in fibroblast proliferation is caused by decreased activity of suppressing tumor proteins, p53. Furthermore, collagen type III concentration was found increased in stage III-IV uterine prolapse without being affected by age and parity. Other studies concluded that collagen type III was a strong risk factor for the occurrence of stage III-IV uterine prolapse.[17,28]

Total collagen concentration was not studied because the focus of this study was to identify the relationship between collagen type III expression on stage III-IV uterine prolapse incidence. Total collagen concentration may be found to decrease or increase in stage III-IV uterine prolapse. The varying study results may be due to some factors, including hormonal status difference of the subject (some received hormonal therapy, some did not). Furthermore, in data analysis, exact stratification to distinguish hormonal influence to total collagen concentration was not possible. Thus, the existing study result could not determine the causal relationship between the variables.[33]

In our opinion, when collagen synthesis activity is increased (especially collagen type III, including immature cross-linking collagen), it makes the sacrouterine ligaments fragile and not strong enough to support pelvic organs. The increase of collagen type III concentration causes a decrease in fibril diameters, thus lowering the strength of sacrouterine ligaments. This indirectly explains the relative decrease of collagen type I to collagen type III. Due to both processes, collagen type I/III ratio decreased in stage III-IV uterine prolapse. Clinically, it can also be used as strong predictor of stage III-IV uterine prolapse.

Low Elastin Expression

Our study found low elastin expression was a risk factor of stage III-IV uterine prolapse as high as 5 times greater (OR 5.40, 95% CI 1.37-21.26; p<0.05) compared to high elastin expression. Elastin level in sacrouterine ligaments relatively decreased in women with stage III-IV uterine prolapse compared to control group. This finding is relevant with majority of existing studies, which found decrease elastin concentration and expression in stage III-IV uterine prolapsed.[18,34] Elastin can be evaluated by various methods, including mRNA expression identification, measuring elastin precursor protein (tropoelastin), indirectly measure its crosslink desmosin, or through optical semi-quantitative analysis as done in this study. But based on various evaluations and methods, the finding was similar: there was a decrease in elastin expression in stage III-IV uterine prolapse compared to control group.

There is multiple underlying mechanism of elastin decrease. First, interaction between genetic factor and habit predisposed one to having stage III-IV uterine prolapse through pelvic supporting ligament stretch mechanism. The stretching activates proliferation and activation of fibroblast secretion.[1] Fibroblast proliferation causes a decrease in extracellular matrix expression. Meanwhile, extracellular matrix expression activity by fibroblast is switched to predominantly a more elastic in nature: collagen

type III, and further increases progressivity of stage III-IV uterine prolapse. Second, increased elastase (substrate that increase elastin degradation) activity is identified. Alpha-1antitrypsin mRNA expression, the inhibitor of serine protease, was found to be decreased in peri urethral tissue of vaginal wall of women with stage III-IV uterine prolapse and stress urinary incontinence.[35] Alpha-1 antitrypsin has been known to be protective to elastin, and the decrease of its expression has been thought to have a role in the increase of elastin degradation in stage III-IV uterine prolapse.

Based on our findings, we recommend: (1) As high estrogen receptor alpha expression and collagen III expression was stage III-IV uterine prolapse risk factors, hormonal replacement therapy can be considered in stage I-II uterine prolapse to prevent the occurrence of stage III-IV, to inhibit the progressivity of uterine prolapse. And eventually, increasing the quality of life of women in the future. (2) A further study is needed to determine the relationship between estrogen receptor alpha expression with low elastin expression on sacrouterine ligaments of patients with stage III-IV uterine prolapse. (3) The knowledge of collagen and elastin as the risk factors of stage III-IV uterine prolapse to be used as a scientific molecular-biological basis for further research on uterine prolapse progressivity prevention.

CONCLUSION

High estrogen receptor alpha and high collagen III expression on sacrouterine ligaments, and a low elastin expression on sacrouterine ligaments were risk factors for stage III-IV uterine prolapse.

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