Research Article

The Estrogen Receptor Concentration and Col3A1 Gene Immunoexpression in Uterosacral Ligament is Correlated with Postmenopausal Uterine Prolapse

Konsentrasi Reseptor Estrogen dengan Imunoekspresi Gen Col3A1 pada Ligamentum Sakrouterinum Berkorelasi dengan Kejadian Prolaps Uteri Pascamenopause

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Abstract

Objective: To analyze correlation between concentration of estrogen receptor and imunoexpression of Col3A1 gene on uterosacral ligament of postmenopausal uterine prolapse patient.

Methods: This is a cross-sectional analytic observational study involving 32 subjects who met inclusion criteria. Samples are taken consecutively in Dr. Hasan Sadikin Hospital in January 1st - December 31st 2012. Statistical analysis was performed with non-parametric Mann-Whitney test and Spearman's rho non-parametric correlation test.

Result: There is no significant difference of estrogen receptor concentration between postmenopausal women with and without uterine prolapse (p = 0.377), while there is no significant difference of Col3A1 gene immunoexpression between postmenopausal women with and without uterine prolapse (p = 0.119) either. There is a significant positive correlation between the estrogen receptor concentration and Col3A1 gene immunoexpression in uterosacral ligament of postmenopausal uterine prolapse patients (p = 0.002, r= 0.711).

Conclusion: There is a positive correlation between the estrogen receptor concentration and Col3A1 gene immunoexpression in uterosacral ligament of postmenopausal uterine prolapse patients.

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Keywords: Col3A1 gene, estrogen receptor, menopause, uterine prolapse, uterosacral ligament

Abstrak

***Tujuan**: Menganalisa korelasi antara kerapatan reseptor estrogen dengan imunoekspresi Gen Col3A1 pada ligamentum sakrouterinum penderita prolaps uteri pascamenopause.*

***Metode**: Penelitian menggunakan metode observasional analitik dengan rancangan cross sectional. Pemilihan 32 sampel yang memenuhi kriteria inklusi secara consecutive sampling selama periode 1 Januari - 31 Desember 2012 di RSHS. Analisis statistik yang digunakan Mann-Whitney dan Spearman.*

***Hasil**: Terdapat perbedaan kerapatan reseptor estrogen yang tidak bermakna pada perempuan pascamenopause dengan dan tanpa prolaps uteri (p = 0,377) dan terdapat perbedaan imunoekspresi gel Col3A1 yang tidak bermakna pada perempuan pascamenopause dengan dan tanpa prolaps uteri (p = 0,119). Terdapat korelasi positif yang bermakna antara kerapatan reseptor estrogen dengan imunoekspresi Gen Col3A1 pada ligamentum sakrouterinum penderita prolaps uteri pascamenopause (p = 0,002) dengan koefisien korelasi (r = 0,711).*

***Kesimpulan**: Terdapat korelasi positif antara kerapatan reseptor estrogen dengan imunoekspresi Gen Col3A1 pada ligamentum sakrouterinum penderita prolaps uteri pascamenopause.*

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***Kata kunci**: gen Col3A1, ligamentum sakrouterinum, menopause, prolaps uteri, reseptor estrogen*

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INTRODUCTION

Pelvic organ prolapse is defined by the International Continence Society as the disruption of structures supporting uterus, bladder, colon, or rectum, which causes one or more of the organs to descend into vagina. According to the report from the 3rd International Consultation on Incontinence (ICI), the prevalence of pelvic organ prolapse (POP) in general population is 5-10%.[1,2] The prevalence is higher in postmenopausal than premenopausal women. This is believed to be caused by the low estrogen level, which leads to down-regulation of estrogen receptors in uterosacral ligament. Ewis in 2004 reported that the low concentration of estrogen receptor is associated with the decline of USL strength and resilience.[3] Estrogen is believed to have a role in the preservation of collagen fibers in connective tissue, especially type 1 collagen which maintain the strength and type 3 collagen maintaining the resilience of connective tissue.[4]

The incidence of uterine prolapse increases with the advancing age, and so does the operative procedures done for uterine prolapse. Weber in 2006 found that the mean age of uterine prolapse patients is 61.5 years old, with more than 50% of them aged 60 or more.[5] The major risk factor of POP are the age and labor process, while the minor risk factors are pregnancy, increasing body mass index, menopause, hypoestrogenism, chronic increase of intraabdominal pressure, trauma, genetic factor, race, musculoskeletal disorders, chronic medical conditions, smoking, and previous surgery.[5-8]

Collagen is the main constituent protein in USL, composing up to 70% of it and supports the integrity of the pelvic organs.[9,10] Any disruption in the renovation of type 1 and type 3 collagen of USL matrix after trauma will affect the normal mechanical function of USL as the supporting structure of pelvic organ anatomy.[11] Connell in 2008 reported a lower transcription of Col1A1 and Col3A1 gene in USL of POP patients compared to those without POP.[12]

Bai in 2005 stated that the low expression of estrogen receptor leads to the decline of estrogenic interstitial effect in the pelvic-supporting connective tissue.[13] Lang in 2003 reported that the decline of estrogen concentration might be related with the occurrence of uterine prolapse.[5] In menopausal age, low concentration of serum estrogen is found, along with the low concentration of estrogen receptor in pelvic-supporting connective tissue.[13]

It is not clearly understood whether the decline of estrogen receptor concentration affects the Col3Al gene expression in USL. Since age is a major risk factor of uterine prolapse, the estrogen deficiency in postmenopausal women is assumed to cause the weakness of uterus-supporting structures (epithelium, connective tissue, and muscle). This is believed to be the key of further development and progression of uterine prolapse, which will explain the increased incidence of uterine prolapse in postmenopausal women. This is seen as an interesting topic, as the relation of estrogen receptor concentration and Col3A1 gene immuno expression in USL of postmenopausal women would be revealed.

METHOD

This is a cross-sectional analytic observational study involving 32 subjects who met inclusion criteria. They were postmenopausal women who had undergone hysterectomy procedure, indicated by POP in 16 subjects and non-POP (epithelial type ovarian carcinoma, stadium I cervical carcinoma, endometrial carcinoma, ovarian cyst, uterine myoma) in the other 16 subjects. All subjects had stage 3-4 uterine prolapse according to POP-Q system, had not received any previous hormonal therapy, and consented to be the study subjects. Subjects were excluded once the microscopic examination showed another pathologic condition in USL, or the USL tissue could not be examined. Samples are taken consecutively in Dr. Hasan Sadikin Hospital in January 1st - December 31st 2012. The specimen examined was paraffin block of the tissue cut from USL. It was taken from the USL part, which was attached to the uterine cervix after hysterectomy indicated by POP, uterine myoma, ovarian carcinoma, cervical carcinoma, and endometrial carcinoma.

The data of each variable was analyzed with Saphiro-Wilk normality test. The homogenously distributed data were then analyzed with non-parametric Mann Whitney test for the one-way hypothesis, and Spearman's rho non-parametric correlation test. P value of <0.05 shows statistical significance of the result.

RESULT

Table 1. Comparison of Estrogen Receptor Concentration and Col3A1 Gene Immunoexpression in USL of Postmenopausal Women with and without Uterine Prolapse.

Variable Expression	with Uterine Prolapse (n=16)	without Uterine Prolapse (n=16)	Statistical Significance*
Estrogen Receptor Concentration (Score)			Z_{M-W} = -0.833
• Median	7.00	6.00	p = 0.377
• Range	3-16	3-12	
Col3A1 Gene (Score)			Z_{M-W} = -1.561
• Median	8.00	12.00	p = 0.119
• Range	1-16	3-16	

*Note: * non-parametric Mann-Whitney test*

Table 1 shows score of estrogen receptor concentration and Col3A1 gene immunoexpression in uterosacral ligament of postmenopausal women with and without uterine prolapse, analyzed with non-parametric Mann-Whitney test. The estrogen receptor concentration is higher in the group with uterine prolapse compared to the group without prolapse, while the Col3A1 gene immunoexpression is lower in the group with uterine prolapse

compared to the group without prolapse. However the differences above are not significant.

Table 2. Correlation between Estrogen Receptor Concentration and Col3A1 Gene Immunoexpression in USL of Postmenopausal Women with and without Uterine Prolapse.

Correlation between Estrogen Receptor Concentration and Col3A1 Gene Immuno expression	r correlation coefficient*	p value*
with uterine prolapse	0.711	0.002
without uterine prolapse	0.228	0.396

*Note: * Spearman's rho non-parametric correlation test*

Table 2 shows that the estrogen receptor concentration is significantly correlated with Col3A1 gene immunoexpression in uterosacral ligament of postmenopausal women with uterine prolapse ($p= 0.002$, $r = 0.711$). Meanwhile, analysis of the correlation done on the group without prolapse does not show a significant result ($p > 0.05$, $r = 0.228$).

DISCUSSION

As shown in Table 2, there is a positive correlation between the estrogen receptor concentration and Col3Al gene immunoexpression in uterosacral ligament of postmenopausal uterine prolapse patients. Consistent with the theory underlying this study, the mechanism of uterine prolapse has not yet been understood. Based on practices, it is proposed that connective tissue structural abnormality or weakness of pelvic-supporting tissue might be the predisposition factor of uterine prolapse. The extracellular matrix is composed of collagen, elastin, and proteoglycan. Stress or pathologic conditions trigger connective tissue to response, thus leads to changes in extracellular matrix.[14]

Bai have reported declined expression of estrogen receptor, progesterone receptor, p53, and p21 in uterine prolapse patients compared to the control population.[5,13] Lang reported that women who experienced POP before menopause had lower concentration of estrogen and estrogen receptor compared to the control group. Furthermore they proposed that the decline of estrogen receptor concentration might be related with the incidence of pelvic organ prolapse.[1]

It is believed that estrogen controls the expression of matrix metalloproteinase (MMPs), which diminishes connective tissue of skin, endometrium, and ovary.[15] Former studies have reported that the expression of matrix metalloproteinase is blocked by estrogen in the uterus-supporting tissue.[16] MMPs contains a proteolytic activity affecting connective tissue proteins such as collagen, proteoglycan, and elastin, therefore impairs the virtual of the extracellular matrix components.[17] Recent study reports HOX gene as the main regulator of organ development, which do so by producing HOX protein, also has an important role in synthesis of type 1 and type 3 collagen fiber of USL matrix in POP.[12]

This study reports a positive correlation between estrogen receptor concentration with Col3A1 gene immunoexpression in uterosacral ligament of postmenopausal uterine prolapse patient. This result indicates that menopause leads to change of estrogen receptor concentration in uterosacral ligament of uterine prolapse patient, drawing signal for Col3A1 gene expression to inhibit the degradation and stimulate synthesis of collagen, so tissue renovation would occur.

This study is consistent with the former theory. The increased score of estrogen receptor concentration and Col3A1 gene immunoexpression in both groups, with positive correlation between the estrogen receptor concentration and Col3A1 gene immunoexpression in uterosacral ligament of postmenopausal uterine prolapse patient, proves that estrogen receptor concentration acts specifically in stimulating collagen synthesis.

This can also be proven on the other collagen marker gene such as Col1A1 gene, HOX gene, or other extracellular matrix components such as elastin, proteoglycan, MMP, and TIMP in uterosacral ligament. Similar studies using other examining parameters such as immunohistochemistry, ELISA, or PCR, is encouraged.

CONCLUSION

There is a positive correlation between the estrogen receptor concentration and Col3A1 gene immunoexpression in uterosacral ligament of postmenopausal uterine prolapse patients.

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