Research Report

Correlation between Content of Collagen I and Tenascin-C Sacrouterine Ligament in the Uterine Prolapse

Korelasi antara Kandungan Kolagen I dengan Tenascin-C Ligamentum Sakrouterina pada Prolapsus Uteri

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Abstract

Objective: To analyze differences in mean and correlation between content of collagen I and tenascin-C sacrouterine ligaments in patients with uterine prolapse.

Method: This type of research is analytic comparative and cross sectional correlation is cut in two research groups. Well conditioned paraffin block of patients uterine prolapse and without uterine prolapse were stained with immunohistochemical staining. The preparation is examined under a light microscope by the Pathology Specialist, assessment of content is done by looking at the distribution and intensity of the color of collagen fibers and tenascin-C.

Result: There were significant mean differences between the content of collagen I and tenascin-C sacrouterine ligament in patients with uterine prolapse and without uterine prolapse (p = 0.001). There was no correlation between the content of collagen I and tenascin-C sacrouterine ligament in patients with uterine prolapse (p = 0.780).

Conclusion: There was a difference in the average content of collagen I and tenascin-C in the uterine prolapse group compared with no uterine prolapse group. There was no correlation between the content of collagen I and tenascin-C in the uterine prolapse group.

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Keywords: collagen I, tenascin-C, sacrouterine ligament, uterine prolapse

Abstrak

***Tujuan**: Untuk menganalisis perbedaan rerata dan korelasi kandungan kolagen I dengan tenascin-C ligamentum sakrouterina pada pasien prolapsus uteri.*

***Metode**: Penelitian ini adalah analitik komparatif dan korelasi secara potong silang pada dua kelompok penelitian. Blok parafin dengan kondisi baik dari pasien prolapsus uteri dan tanpa prolapsus uteri diwarnai dengan pewarnaan imunohistokimia. Sediaan diperiksa di bawah mikroskop cahaya oleh Spesialis Patologi Anatomi, penilaian kandungan dilakukan dengan melihat distribusi dan intensitas warna serat kolagen dan tenascin-C.*

***Hasil**: Terdapat perbedaan rerata yang bermakna antara kandungan kolagen I dan tenascin-C ligamentum sakrouterina prolapsus uteri dan tanpa prolapsus uteri (p = 0,001). Tidak ada korelasi antara kandungan kolagen I dan tenascin-C ligamentum sakrouterina pada pasien prolapsus uteri (p = 0,780).*

***Kesimpulan**: Terdapat perbedaan rerata kandungan kolagen I dan tenascin-C pada kelompok prolapsus uteri dibanding tanpa prolapsus uteri. Tidak terdapat korelasi antara kandungan kolagen I dengan tenascin-C pada kelompok prolapsus uteri.*

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***Kata kunci**: kolagen I, tenascin-C, ligamentum sakrouterina, prolapsus uteri*

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INTRODUCTION

The aging of population has led to increase incidence of Pelvic Organ Prolapse (POP) in developed and developing countries, nearly 11% of the entire population of women reaching the age of 80 years will have a risk for uterine prolapse or urinary incontinence and one third of them will undergo surgery.[1-3]

One form of Pelvic Organ Prolapse is a uterine prolapse. Decrease in uterine prolapse is a condition of the uterus into the vagina or out of the vagina due to the weakening of the connective tissue of the uterus backer. Uterine prolapse is a disease that is widely available in the community and its incidence increases with age and parity.[4,5]

Gregory et al[6] of 2005 said that the incidence of uterine prolapse varies in a particular race. Spanish Caucasian race have a higher risk than Asian, African, and Indian races. According to the annual report of Obstetrics and Gynecology RSHS 2006, from 1455 gynecological cases treated there were 30 cases of uterine prolapse and 13 cases in which hysterectomy vaginal.[7]

Some important factors in the incidence of uterine prolapse are the age factor, the amount of labor, delivery the number of vaginal delivery and obesity. Especially when the process occurs in one of the most important part of the pelvic support system such as the cardinal ligament or sacrouterine ligament or because of damage to the pelvic diaphragm and urogenital.[8,9]

Sacrouterine ligament is the ligament that holds the uterus in order not to move around, bring back the cervical curve of the left and right through the rectal wall toward the left and right os sacrum. In vitro studies showed that the ligament can withstand more load than sacroutrtine 17 kg. Important element of the stability of this network is the quantity, structure and

regulation of extracellular matrix proteins such as elastin, collagen and fibronectin and its receptor as well as integrin.[10,11]

Connective tissue is formed by fibroblast cells and extracellular matrix. The content of the extracellular matrix is divided into two main proteins that form connective tissue, ie collagen and elastin. Extracellular matrix also contains glycosaminoglycans such as tenascin, fibronectin and laminin. Collagen and elastin determine the strength of the network, and glycoproteins such as tenascin plays a role in cell adhesion to collagen, wound healing and the tumor process.[9-12]

The relationship between the content of collagen and tenascin-C with the incidence of uterine prolapse and its comparison with patients without uterine prolapse still little has data. The content of tenascin-C in humans is found in the central nervous system, smooth muscle and ligament. Tenascin-C is a protein thought to play a role in the process of cell adhesion to collagen fibroblast.[13-15]

The purpose of this study was to analyze the mean and the correlation between the content of collagen I and tenascin-C sacrouterine ligament in uterine prolapse patients.

METHODS

Sacrouterine ligament was identified with the discovery of macroscopic fiber that extends from the sacrum to the cervical or vaginal apex along the pelvic sidewall. Sacrouterine ligament along 1 cm of the cervix was taken and then the samples were fixed in formalin 10% and put in a paraffin block and then made preparations stained with immunohistochemistry on outbond on outer appearance of collagen I antibody (ab90395) and tenascin-C antibody (ab6346) from Abcam. The preparation was examined under a light microscope by the Pathology Specialist, assessment of content was done by looking at the distribution and intensity of the color of collagen fibers and tenascin-C.

Data analysis was performed using SPSS version 18.0 for Windows. Significance of test results was determined by the value of $p \leq 0.05$.

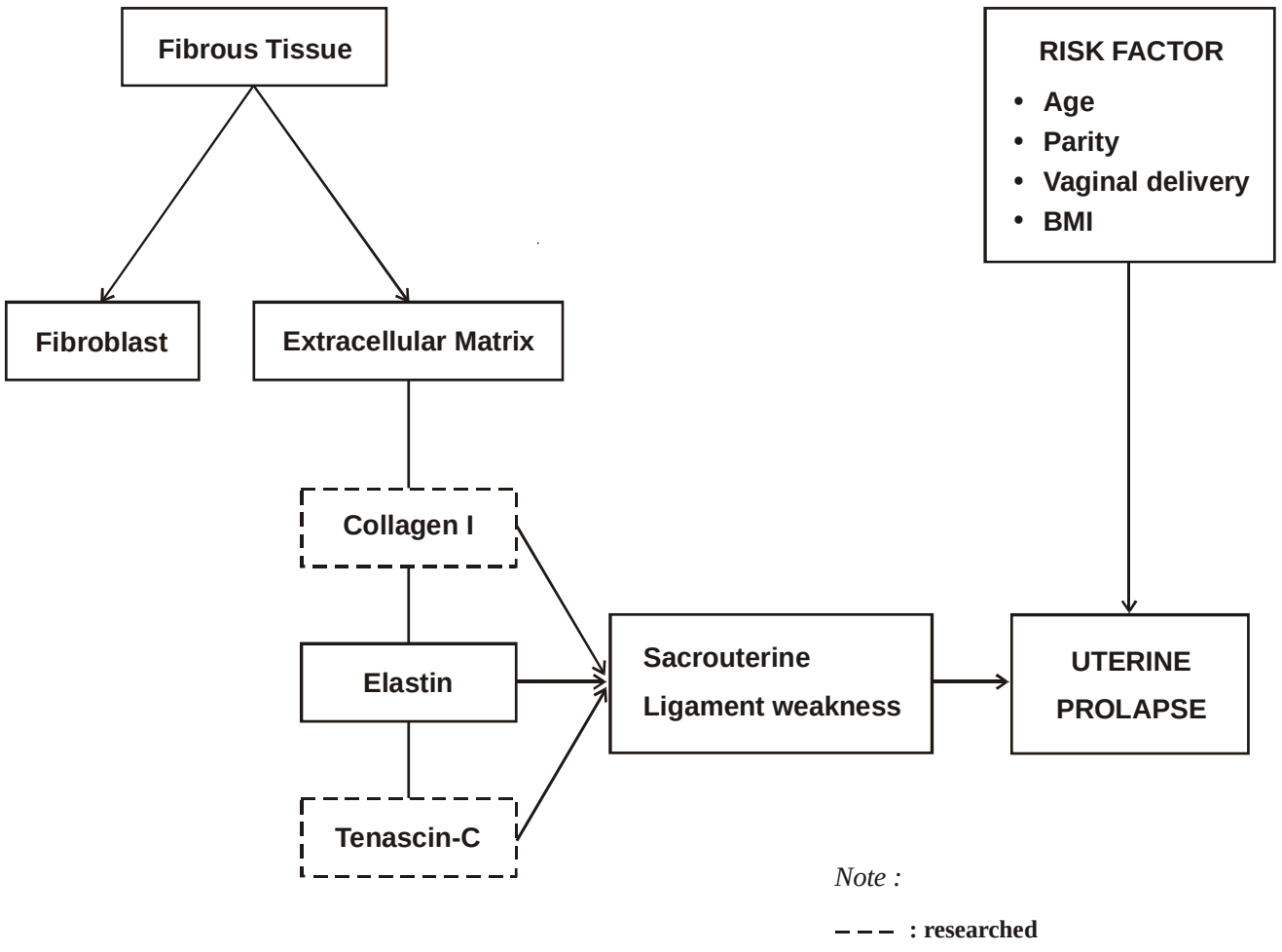

Figure1. Conceptual Framework

RESULT

Table 1. Research Subject Characteristics.

Characteristics	Research Groups		p < 0.05
	Uterine Prolapse (n=16)	Control (n=16)	
Age			
• Mean (SD)	51.94 (4.25)	49.18 (3.95)	0.068
• Median	53	49.5	
• Range	44 - 58	41 - 55	
Parity			
• Mean (SD)	3 (0.85)	2.7 (1.06)	0.395*
• Median	3.00	3.00	
• Range	1 - 4	1 - 4	
Vaginal Delivery			
• Mean (SD)	3.37 (0.95)	3.12 (0.88)	0.449
• Median	3.00	3.00	
• Range	2 - 5	2 - 5	
Body Mass Index			
• Mean (SD)	21.95 (1.79)	23.32 (2.17)	0.054*
• Median	21.35	23.30	
• Range	20 - 26.84	20 - 29.28	

Note: Statistics by unpaired t test
**Statistics by Mann Whitney test*

Table 2. Differences in the average content of collagen I and tenascin-C sacrouterine ligament in patients with and without uterine prolapse.

		Uterine Prolapse (n=16)	Non Uterine Prolapse (n=16)	p
Collagen I	Mean	6.94	12.25	0.001
	Median	7	12	
	Range	3 - 9	9 - 16	
Tenascin-C	Mean	12.88	7.82	0.001
	Median	12	9	
	Range	9 - 16	4 - 9	

Note: P value calculated by Mann Whitney test

Table 3. The correlation between the content of collagen I and tenascin-C sacrouterine ligament in patients with uterine prolapse

	Uterine Prolapse (n=16)	Non Uterine Prolapse (n=16)
Corellation Coefficient (r)	0.076	- 0.469
P value	0.780	0.067

Note: r = Corellation Coefficient Spearman

DISCUSSION

This study compared the characteristics of research subjects consisted of age, parity, number of vaginal birth and body mass index. This was stated by the idea that age, parity, number of vaginal birth, and body mass index are risk factors that will affect the incidence of uterine prolapse. Connective tissue and fascia of the organs in the pelvic floor may lose power due to the aging process or loss of neuroendocrine signals. The highest incidence of POP in the postmenopausal period is allegedly due to the decrease of hormone estrogen.[16-17] Karam et al[18] in his study only focused on a group of postmenopausal women; minimized selection bias by assessing the research group. Uterine prolapse and control group had the same basic characteristics, such as age, parity, number of vaginal birth, BMI, use of hormone therapy and smoking status. It was possible to isolate the association of collagen I with POP, with minimal confounding effects of other variables that are important causes. Table 1 showed the characteristics of both study groups. After comparing the characteristics of research subjects, there was no average age difference between the study group with uterine prolapse and group without uterine prolapse. Statistical test results showed no significant difference between age of patients with uterine prolapse and patients without uterine prolapse. In the uterine prolapse group were found the number of parity, the number of vaginal birth and relative body mass index as compared with the group without uterine prolapse.

The first hypothesis that there is a difference between the average levels of collagen I and without uterine prolapse. This hypothesis was tested by Mann Whitney test p = 0.001 values were obtained, since the value of p < 0.05 is significant or has meaningful research results (H0 is rejected). This means that there are differences between the mean levels of collagen I and without uterine prolapse. The results of this study showed that a mean collagen I in patients with uterine prolapse was 6.94, lower than in patients without uterine prolapse which 12.25. This is consistent with previous studies stating that in patients with uterine prolapse I was found a decreased collagen content. Collagen I as one of the main components of the extracellular matrix of sacrouterine ligament sacrouterine underwent a process of degradation caused by stress.

The second hypothesis is that there are differences in mean levels of tenascin-C between uterine prolapse and no prolapse uteri. This hypothesis was tested by Mann Whitney test and the values obtained for p = 0.001 p value < 0.05 is significant or has meaningful research results (H0 is rejected). This means that there are differences in mean levels of tenascin-C between uterine prolapse and no prolapse uterine.

The results of this study showed a mean tenascin-C in patients with uterine prolapse was 12.88, higher than in patients without uterine prolapse which was 7.82. The results are consistent with previous studies conducted by Ewies et al 15 which showed that tenascin expression was significantly higher in the prolapse regardless of menopausal status. In the non-prolapse group, the percentage of tenascin staining was also significantly higher after menopause. Tenascin-C as one of the main components of the extracellular matrix of sacrouterine ligament had increased production caused by stress.

In vivo, the majority of cultured cells were expressing tenascin-C when exposed to growth factors or connect hormones. For example, fibroblasts was expressing tenascin-C in response to factors associated with inflammation and wound healing, which includes TGF-β, basic fibroblast growth factor and interleukin-1.[19]

Speed ligament remodeling in patients affected POP biochemical changes in the extracellular matrix such as collagen, elastin and adhesive glycoproteins such as tenascin-C. Miofibroblast plays an important role of extracellular matrix remodeling and is regulated by the regulator such as transformation growth factor (TGF)-β, trombospondin (TSP) 1 and matrix metalloproteases (MMPs). Impaired balance between protein synthesis and damage to the pelvic floor's extracellular matrix cause POP.[20]

Based on statistical analysis in Table 3 looks very weak correlation between the content of collagen I and tenascin-C sacrouterine ligament in patients with uterine prolapse. The mechanism of uterine prolapse is not clear until recently, is thought to change the content of the extracellular matrix in the ligament as a cause. Extracellular matrix is composed of collagen, elastin, and proteoglycans. Conditions of stress such as parturition, obesity, and age leads to changes in the content of the extracellular matrix.

Sacrouterine ligament consists of collagen fibers that give strength when stretched. Ligament contains 70-80% of collagen, predominantly type I collagen, the main fibers are resistant to stress, are patches of collagen III, V and VI.[15]

Ayman et al reported a significant increase in tenascin content in th suffered uterine prolapse ligament regardless of menopausal status. Tenascin is a large glycoprotein that has transients in the extracellular matrix and is involved in morphogenetic movement and tissue regeneration and tumor. This process is generally found in adult tissue remodeling such as experienced wound healing. The finding of increased tenascin in the ligament that had prolapse can be viewed as a form of tissue trauma.

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This study did not prove the existence of a correlation between the content of collagen I and tenascin-C in patients with sacrouterine ligament uterine prolapse. Over the years of research on protein tenascin-C is expected this, as other extracellular matrix glycoproteins, will increase the adhesion of cells. Several studies investigating the role of tenascin-C as an adhesive protein proved that this protein does not have the same effect on cells as well as the classic adhesive macromolecules. There are discrepancies in the literature as to whether tenascin-C and allowing of the attachment of these substances seem to inhibit the adhesion of cells to matrix proteins such as fibronectin and laminin others. Tenascin-C is the corresponding that has been allocated to one group of proteins called "anti-adhesive protein". Now there is general agreement that some type of action of cells attached to tenascin-C are specific, but weak in adhesion. The cells attached to tenascin-C can not have a visible cytoskeletal rearrangement in cells attached to fibronectin. Proteins is a strong adhesive substrate for epithelial cells in her basal membrane, or fibro-blasts in collagen environment. Tenascin-C that allows only a weak adhesive, is impossible to play this particular mechanic.

CONCLUSION AND SUGGESTION

The results of this study was that there were differences in mean of collagen I and tenascin-C in patients with sacrouterine ligament uterine prolapse compared with patients without uterine prolapse. There is no correlation between collagen I and tenascin-C in patients with sacrouterine ligament uterine prolapse.

This research can be continued with more number of samples with cohort design so that expected results can be statistically significant.

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