

Research Article

Tenascin-C is Strongly Expressed in the Anterior Vaginal Walls of Women with Pelvic Organ Prolapse

Tenascin-C Diekspresikan dengan Kuat pada Dinding Vagina Anterior Perempuan dengan Prolaps Organ Panggul

Risma N Sulham, Trika Irianta, Umar Malinta

Department of Obstetrics and Gynecology

Faculty of Medicine University of Hasanuddin/Dr. Wahidin Sudirohusodo Hospital
Makassar

Abstract

Objectives: To compare the expression of tenascin in the anterior vaginal wall in women with pelvic organ prolapse and without pelvic organ prolapse (POP).

Method: This study was a cross sectional study. The study was conducted in RS Dr. Wahidin Sudirohusodo and other network hospitals of Obstetrics and Gynecology Department of Hasanuddin University, Makassar, from 1st January 2011 to 1st April 2012. This study assessed the expression of tenascin in 35 women with pelvic organ prolapse grade III and IV and the controls were 35 women without pelvic organ prolapse. Tenascin expression was assessed by immunohistochemical examination using tenascin antibody staining (mouse monoclonal antibody Novacastratenascin C, code NCL-TenasC). Sampling was done by consecutive sampling. Data processed by the chi-square. The significance level used was 0.05.

Result: The result of this research shows that with exception of history of bearing baby > 4000 gr weight ($p=0.572$); age, parity, menopausal status, and body mass index were significantly different between the group. This indicates that the variable does not affect the calculation of research data. While parity, menopausal status and body mass index (BMI), from an analysis of risk factors for POP were associated with increased intensity of tenascin is significant in postmenopausal status, and BMI > 25 (overweight) ($p < 0.05$). Expression of tenascin in a sample of moderate (+ +) as many as 18 cases (51.5%) compared with controls expression of tenascin weaker (+) 25 cases (71.5%).

Conclusion: Expression of tenascin robust anterior vaginal wall in women with POP and weak women without POP.

[Indones J Obstet Gynecol 2013; 1-4: 188-93]

Keywords: pelvic organ prolaps, risk factors, tenascin-C

Correspondence: Risma N Sulham. Jln.Toddopuli VI C 2B, Makassar. Telephone/mobile: 08114109330, (0411)2592006
email:rismasulham@yahoo.com

Abstrak

Tujuan: Membandingkan ekspresi tenascin dinding vagina anterior pada perempuan dengan prolaps organ panggul dan tanpa prolaps organ panggul (POP).

Metode: Penelitian ini adalah penelitian potong lintang. Penelitian ini dilakukan di BLU RS Dr. Wahidin Sudirohusodo dan beberapa rumah sakit pendidikan bagian obstetric dan ginekologi FK-UNHAS yang dimulai pada tanggal 1 Januari 2011 sampai 1 April 2012. Penelitian ini menilai ekspresi tenascin pada 35 perempuan dengan prolaps organ panggul tingkat III dan IV dan sebagai kontrol adalah 35 perempuan tanpa prolaps organ panggul. Ekspresi tenascin dinilai dengan pemeriksaan imunohistokimia menggunakan pewarnaan antibodi tenascin (Novacastra mouse monoclonal antibody tenascin C, code NCL-Tenas C). Pengambilan sampel dilakukan secara consecutive sampling. Data diolah dengan chi-square. Tingkat signifikansi yang digunakan adalah $p=0,05$.

Hasil: Hasil penelitian menunjukkan bahwa karakteristik subjek penelitian berdasarkan usia, pendidikan, paritas, riwayat persalinan, riwayat melahirkan bayi besar, tidak bermakna secara statistik. Hal ini menunjukkan bahwa variabel tersebut tidak mempengaruhi perhitungan data penelitian. Sedangkan karakteristik status menopause dan Indeks Masa Tubuh (IMT), dari analisis faktor risiko terjadinya POP yang dihubungkan dengan peningkatan intensitas tenascin tampak signifikan pada status postmenopause dan IMT > 25 (overweight) ($p < 0,05$). Ekspresi tenascin pada sampel moderat (++) sebanyak 18 kasus (51,5%) dibandingkan dengan kontrol ekspresi tenascin yang lebih lemah (+) sebanyak 25 kasus (71,5%).

Kesimpulan: Ekspresi tenascin-C dinding vagina anterior kuat pada perempuan dengan POP dan lemah pada perempuan tanpa POP.

[Maj Obstet Ginekol Indones 2013; 1-4: 188-93]

Kata kunci: faktor risiko, prolaps organ panggul, tenascin-C

INTRODUCTION

Pelvic organ prolapse (POP) is a morbid condition which affects the quality of women life. It has a high prevalence in developed countries, although the major etiology is different.¹ Failure of the normal function of levatoranimuscle plays an important role in the incident of pelvic organ prolapse in women.² The incidence of pelvic organ prolapse on the anterior wall of vagina was 9.3 cases per 100

women per year, on the posterior wall of the vagina about 5.7 cases per 100 women per year, and uterine prolapse about 1.5 cases per 100 women per year.³

Little is known about the characteristics of histomorphology of the vaginal wall and the changes in the connective tissue in women with pelvic organ prolapse. Connective tissue consists of collagen, elastic fibers and proteoglycans as a major compo-

ment of the extracellular matrix. The main elements of network stability are quantity, ultrastructure and organization of protein-extracellular matrix such as tenascin, elastin, collagen and fibronectin and their receptors, such as integrins.⁴

Tenascin is a large glycoprotein, which presents in the extracellular cells of and involved in morphogenetic movements, tissue pattern formation, and tissue repair.⁵ The findings of increased tenascin in the ligament prolapse acceptable due to pelvic organ prolapse can be regarded as a form of tissue trauma. Increased tenascin expression in the vaginal wall indicates involvement in the healing process after surgery.⁶

Several studies have focused on the role of connective tissue and collagen in incidence of posterior vaginal wall prolapse among women.⁷ Delancey (1992) suggested that the vaginal epithelium, collagen and smooth muscle layers deeper to provide support along the walls of the vagina and may be more susceptible to secondary effects of prolapse.⁸ Overall, the decrease in immune reactivity increased synthesis of elastin and tenascin may explain the loss of function of the connective tissue supporting the uterus. These data indicate the possible role of these proteins in the pathogenesis of pelvic organ prolapse as well as provide an overview of treatment. The research that support the abnormality structure of connective tissue or repair mechanism which may predispose women to suffer from pelvic organ prolapse has not been done in Indonesia, so with that background, the research was conducted.

METHODS

This was a cross-sectional study. The data was processed using SPSS for windows version 17. Chi-Square or Fisher Exact test and Mann Whitney U-test was used to compare values of nominal and scale variable from two or more unpaired groups. In this case, we compare the expression of tenascin in pelvic organ prolapse grade III and IV with women without pelvic organ prolapse.

The research was conducted in the teaching hospital of obstetrics and gynecology Medical Faculty of Hasanuddin University, Dr. Wahidin Sudirohusodo Hospital, and other network hospitals as well as laboratory examination of pathology Anatomy Faculty of Medicine Hasanuddin University. This study was carried out from January 2011 - April 2012.

The study population was women with pelvic organ prolapse grade (III and IV) who were hospitalized and underwent surgery. Samples were women with severe pelvic organ prolapse grade (III and IV).

Consecutive sampling conducted, that is, all subjects who meet the criteria for inclusion in the study were selected. All patients had been provided informed consent before participating as research subjects. The sample size and the desired level of accuracy as well as the relative provisions in accordance with the table of Isaac and Michael.

From this study, a sample of 35 cases of uterine prolapse grade III and IV, which carried a total of transvaginal hysterectomy act as a case group, and 35 cases of benign gynecological perabdominal total hysterectomy as a control group.

Data analysis was performed with Mann-Whitney test to compare the expression of tenascin between the prolapse and without pelvic organ prolapse, Chi-Square test and Fisher Exact (if the expected value of the number of cells in the table exceeds 20%) to assess homogeneity of characteristics (age, parity, history of surgery, and BMI) between the two groups, and the level of significance used was 0.05 (considered significant if $p < 0.05$).

RESULTS

In this study, we collected 35 cases of pelvic organ prolapse grade III and IV, and 35 cases of benign gynecological tumor as the control group. In Table 1 it appears that the characteristics of patients most at age > 45 years both in the case of pelvic organ prolapse and in control. There was no significant difference in the average age between the two groups ($p > 0.05$).

The group of pelvic organ prolapse is the highest group who has high level of education = 9 years as many as 26 people (74.3%), while group without pelvic organ prolapse as many as 26 people (74.3%). This difference was not statistically significant ($p > 0.05$).

Most parity on pelvic organ prolapse group is the child > 3, as many as 28 cases (80.0%), similar to the group without pelvic organ prolapse is children > 3 as many as 19 cases (54.3%). This difference was not statistically significant ($p > 0.05$).

Table 1. The Characteristics of Women with and without Pelvic Organ Prolapse

Characteristics	Total				p Value
	With Prolapse		Without Prolapse		
	n	%	n	%	
Age (Years)					
≤ 45	2	5.7	2	5.7	0.641
> 45	33	94.3	33	94.3	
Education (Years)					
≤ 9	26	74.3	26	74.3	0.767
> 9	9	25.7	9	25.7	
Parity					
0	2	5.7	4	11	0.072
1 - 2	5	14.3	12	34.3	
> 3	28	80.0	19	54.3	
Delivery History					
Nullipara	2	5.7	4	11.4	0.367
Normal	32	29.4	27	77.1	
Vacuum	1	2.9	0	0	
Sectio Caesarian	0	0	4	11.4	
Delivery history of large baby					
≤ 3500 gram	28	80	30	85.7	0.376
> 3500 gram	7	20	5	14.3	
Menopausal Status					
Premenopause	4	11.4	21	60	0.003
Menopause	31	88.6	14	40	
BMI					
Normal 18.5-24.99	11	3.4	22	62.9	0.008
Obesity > 25.0	24	68.6	13	37.1	

Chi-square test, Mann-Whitney $p = 0.000$ ($p < 0.05$)

Types of labor history at prolapse group with a history of pelvic organs are normal delivery ie 32 cases (9.4%), while in the group without pelvic organ prolapse as many as 27 cases (77.1%). This difference was not statistically significant ($p > 0.05$).

In the group of pelvic organ prolapse 28 cases (80.0%) had a history of having a baby > 3,500 grams, while in the group without pelvic organ prolapse as many as 30 cases (85.7%) who had delivered babies over 3,500 grams. However, this difference was not statistically significant ($p > 0.05$).

In the group of pelvic organ prolapse as many as 31 cases (88.6%) had experienced menopause, being in the group without pelvic organ prolapse were 21 cases (60%) have not experienced menopause. This difference was statistically significant ($p < 0.05$).

In the group of pelvic organ prolapse as many as 24 cases (68.6%) have the BMI > 25, while in

the group without pelvic prolapse were 22 cases (62.9%) had a normal BMI, and only 13 cases (37.1%) who had BMI > 25. This difference was statistically significant ($p < 0.05$).

In Table 2 indicates that there are differences in the expression of tenascin in both cases and controls. Results of Mann-Whitney test to compare between the two groups showed that in cases of pelvic organ prolapse, weak expression of tenascin (+) there were 11 cases (31.4%), moderate expression (+ +) of 18 cases (51.5%) and expression of tenascin strong (+ + +) was 6 cases (17.1%). When compared with the group without pelvic organ prolapse, the expression of positive tenascin note with a weaker expression. In the control group obtained a weak expression (+) of 25 cases (71.5%), moderate expression of 9 cases (25.7%) and strong expression (+ + +) 1 (2.8%).

Table 2. Tenascin Expression of Anterior Vaginal Wall among Women with and without Pelvic Organ Prolapse

Tenascin Expression	Group		Total
	POP	Without POP	
Mild	11	25	36
	31.4%	71.5%	51.5%
Moderate	18	9	27
	51.5%	25.7%	38.5%
Strong	6	1	7
	17.1%	2.8%	10%
Total	35	35	70
	100%	100%	100%

$p = 0.000$ ($p < 0.05$)

In Table 3, menopausal status was also one of significant risk factor for the occurrence of pelvic organ prolapse which is associated with increased expression of tenascin. In the group of premenopausal pelvic organ prolapse, we found strong expression of tenascin (+ + +) in 1 case, moderate (+ +) about 2 cases and weak (+) 1 case. On postmenopausal women we found strong expression (+ + +) 17 cases, moderate (+ +) 12 cases and weak (+) of 2 cases. In contrast to the case without pelvic organ prolapse in premenopausal women, it showed weak expression of tenascin (+) of 16 cases, moderate (+ +) of 4 cases and strong (+ + +) only 1 case. In the case without pelvic organ prolapse of postmenopausal women, we found a weak expression of tenascin (+) 11 cases, moderate (+ +) as much as 3 cases, and found no cases with strong tenascin expression (+ + +).

Table 3. Risk Factor Analysis of Pelvic Organ Prolapse

Risk Factor	Tenascin Expression						p
	With POP			Without POP			
	Weak (+)	Moderate (++)	Strong (+++)	Weak (+)	Moderate (++)	Strong (+++)	
Age (Years)							0.641
≤ 45	0	1	0	0	0	1	
> 45	3	13	18	27	7	0	
Education (Years)							0.767
≤ 9	3	11	12	22	7	0	
> 9	0	3	6	3	3	0	
Parity							0.072
0	0	1	1	5	0	0	
1 - 2	0	2	3	7	4	1	
> 3	3	11	14	16	2	0	
Delivery History							0.367
Nullipara	0	1	1	4	0	0	
Normal	3	13	16	19	7	1	
Vacuum	0	0	1	0	0	0	
SC	0	0	0	4	0	0	
Children Birthweight							0.75
≤ 3500 gram	3	12	13	23	6	1	
> 3500 gram	0	3	4	4	1	0	
Menopausal Status							0.001
Premenopause	1	2	1	16	4	1	
Menopause	2	12	17	11	3	0	
BMI							0.041
Normal 18.5 - 24.99	2	2	6	7	3	1	
Obesity > 25.0	1	13	11	10	1	3	

From the analysis of body mass index (BMI), BMI ≥ 25 (overweight) was considered as a significant risk factor for the occurrence of pelvic organ prolapse and was also associated with an increased intensity of tenascin. In the group of pelvic organ prolapse with normal BMI, we found strong expression of tenascin (+ + +) in 6 cases, moderate (+ +) and 2 cases of weak (+) 2 case. In the obese women found strong expression (+ + +) 11 cases, moderate (+ +) as many as 13 cases and weak (+) 1 case. In contrast to the case without pelvic organ prolapse in the normal BMI, showed weak expression of tenascin (+) 7 cases, moderate (+ +) of 3 cases and strong (+ + +) only one case. In the case without pelvic organ prolapse obese women found weak expression of tenascin (+) 10 cases, moderate (+ +) 1 case, and strong expression of tenascin (+ + +) of 3 cases.

DISCUSSION

Characteristics of the age group with and without prolapse reveal the same that is over 45 years old. In this study, no significant association, but was positively related due to increased expression of tenascin in the age group over 45 years.

Mac Lennan et al (2000) concluded that the pelvic muscles and pelvic organs ancillary network tend to decrease in strength along with age. This is a factor that cannot be avoided.¹

Research by Swift et al (2000) showed that pelvic organ prolapse grade I or II occurs mostly in younger women. Pelvic organ prolapse grade III and IV of 2.6% turned out to be the most at age > 40 years and the prevalence increased to 21% in women aged > 70 years. In this study it was found that there was no difference in age between women with prolapse grade III, IV and women

without pelvic organ prolapse, but age and tenascin expression was positively related due to increased tenascin expression in pelvic organ prolapse over the age of > 45 years.⁹

Most subjects in both groups had lower education (≤ 9 years). In this study the majority of the sample were housewives with low levels of education, in which the characteristics of the group of pelvic organ prolapse and pelvic organ prolapse showed non-homogeneous or uniform picture.

Parity was found to be not significant ($p > 0.05$). In this study, we found 28 patients (80%) in the case group with history of childbirth of more than three times; there were 19 patients (54.3%) history of childbirth of more than three times in the control group. Even if it was not statistically significant, it could be seen that the incidence of pelvic organ prolapse was higher in patients with history of childbirth of more than three times.

This is according to research conducted Chiafarino F et al. who compared between nulliparous and women with high parity. Women with higher parity have a higher risk for the occurrence of genital prolapse (OR 2.6) compared with women who had never given birth, where OR 3.0 for women with one vaginal delivery, and 4.5 for women with 2 or more births vaginal.¹⁰ The foregoing supports the hypothesis that vaginal delivery results in significant tissue damage proponent which can lead to weakening of the pelvic floor muscles and pudendal nerve in all women.

History of delivery between the two groups was not statistically significant. In this study, the incidence of pelvic organ prolapse in patients who underwent vaginal delivery is higher compared with patients with vaginal delivery without pelvic organ prolapse.

The study found no significant association between infant birth weight > 3,500 g with < 3,500 grams. But the weight of the baby, had a role on the occurrence of pelvic organ prolapse, which in this study found that the incidence of pelvic organ prolapse was higher in infants weighing > 3,500 compared with < 3,500 grams.

The results of this study showed that the anterior vaginal wall in patients with pelvic organ prolapse is characterized by the higher presence of an immune reaction to tenascin compared to patients without pelvic organ prolapse. Tenascin expression in women with moderate pelvic organ prolapse of

+ 2 were found in 18 cases (51.5%), while in women without pelvic organ prolapse with a weaker expression of tenascin is +1 as many as 25 cases (71.5%). This is consistent with research on immune tenascin labeling in the anterior vaginal wall in women after menopause with and without pelvic organ prolapse which has been done by Goepel in 2007 involving 59 women (average age 59-82) who underwent surgery in the Department of Gynecology, Martin-Luther-University Halle-Wittenberg.¹¹ The results showed that vaginal wall of prolapse patient is characterized by the presence of a strong immunolabel whereas in patients without vaginal wall prolapse, the immunolabel was weaker than prolapse patients.

Tenascin is involved in adhesion, migration, cell growth and wound healing as well as neovascularization. Tenascin may be involved both in physiological or pathological functions, depending on the circumstances and the type of cell as well as the developmental grade of the organism. The dramatic changes also happened in various pathological conditions such as tumor, tendon degeneration, synovitis, colitis, colon adenoma and colorectal carcinoma, pathological bone marrow and interstitial pneumonia.

The pattern of expression changes in tenascin expression in the anterior vaginal wall at the prolapse occurs because prolapse is a healing phase in tissue trauma. Trauma itself may have been initiated by various events such as delivery. Lack of estrogen after menopause is also likely to have led to decompensated network.¹¹

Pathophysiology of pelvic organ prolapse in patients with obesity is the occurrence of increasing intra-abdominal pressure, resulting in suppression of the pelvic wall. The pressure of pelvic wall causes vascularization of the pelvis become worst and later decrease the muscle tone.

CONCLUSIONS

The expression of tenascin-C in the anterior vaginal wall among women with pelvic organ prolapse grade III, IV is stronger than women without pelvic organ prolapse. In this study, the BMI and menopausal status obtained statistically significant. Further research needs to be done at the molecular level to investigate the pathogenesis of tenascin in pelvic organ prolapse. This research is expected to reduce the incidence of pelvic organ prolapse by

providing counseling to the patients with predisposing factors for pelvic organ prolapse.

REFERENCES

1. MacLennan A. The descent of Woman. *Climacteric* 2000; 3:225-6.
2. Boreham MK, Way CY, Miller RT, Schaffer JI, Word RA. Morphometric analysis of smooth muscle in the anterior vaginal wall of women with pelvic organ prolapsed. *Am J Obstet Gynecol* 2002; 187:56-63
3. Amundsen CL, Flynn BJ, Webster GD. Anatomical correction of vaginal vault prolapsed by uterosacral ligament fixation in women who also required a pubo vaginal sling. *J Urol* 2003; 169:1770-4
4. Ulmsten U, Falconer C. Connective tissue in female urinary incontinence. *Curr Opin Obstet Gynecol* 1999; 11:509-15
5. Liu S, Yang R, Al-Shaikh R, Lane J. Collagen in tendons, ligaments and bone healing. *Clin Orthopaedy* 1995; 318:265-78
6. Neurath M. Expression of tenascin, laminin and fibronectin following traumatic rupture of the anterior cruciate ligament. *Z Orthop* 1993; 131:168-72
7. Norton P, Boyd C, Deak S. Collagen synthesis in women with genital prolapsed or stress urinary incontinence. *Neurol Urogyn* 1992; 11:300-1
8. Delancey JOL. The hidden epidemic of pelvic floor dysfunction: Achievable goals for improved prevention and treatment. *Am J Obstet Gynecol* 2005; 192:5
9. Swift SE, Woodman P, O'Boyle AL. Pelvic Organ Support Study (POSST): The distribution, clinical definition, and epidemiologic condition of pelvic organ support defects. *Am J Obstet Gynecol* 2005; 192:3
10. Chiaffarino F. Reproductive factors, family history, occupation and risk of urogenital prolapse. *Eur Obstet Gynecol J* 1999; 82: 63-7
11. Goepel Christian. Differential elastin and tenascin immune labeling in the utero sacral ligaments in postmenopausal women with and without pelvic organ prolapsed. *Elsevier* 2007; 110:204-9