Research Article

Transforming Growth Factor β1 and Tropoelastin Expression in Uterine Prolapse

Ekspresi Transforming Growth Factor β1 dan Tropoelastin pada Prolaps Uteri

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Abstract

Objective: To know the correlation of the expression of transforming growth factor beta (TGF-β1) and tropoelastin in uterine prolapse.

Method: A cross-sectional study of 30 subjects suffered from uterine prolapse in the Department of Obstetrics and Gynecology Dr. Mohammad Hoesin hospital Palembang. The study was conducted since December 1st, 2014 until July 31st, 2015. The sample was from the sacrouterine ligament and immunohistochemical examination was conducted to see the expression of TGF-β1 and tropoelastin.

Result: Of the 30 subjects obtained, the expression of TGF-β1 was on 30 subjects consisting of 18 (60%) for weak expression and 12 (40%) for strong expression. Meanwhile, the strong tropoelastin expression was on 18 subjects (60%) and weak tropoelastin expression on 12 subjects (40%). There was a positive correlation between TGF-β1 and tropoelastin expression with moderate correlation (p=0.014; r=0.44).

Conclusion: There is a positive correlation between the TGF-β1 and tropoelastin expression of sacrouterine ligament in uterine prolapse with moderate correlation.

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Keywords: transforming Growth Factor Beta 1, tropoelastin, uterine prolapse

Abstrak

***Tujuan**: Untuk mengetahui korelasi ekspresi transforming growth factor beta 1 (TGF-β1) dengan ekspresi tropoelastin pada prolaps uteri di Rumah Sakit Dr. Mohammad Hoesin Palembang.*

***Metode**: Sebuah penelitian potong lintang dari 30 subjek penderita prolaps uteri yang dilakukan di Departemen Obstetri dan Ginekologi Rumah Sakit Dr. Mohammad Hoesin Palembang. Penelitian dilakukan sejak 1 Desember 2014 sampai dengan 31 Juli 2015. Sampel berasal dari ligamentum sakrouterina dan dilakukan pemeriksaan imunohistokimia untuk melihat ekspresi TGF-β1 dan tropoelastin.*

***Hasil**: Dari 30 subjek didapatkan ekspresi TGF-β1 kuat pada 12 (40%) subjek, ekspresi TGF-β1 lemah pada 18 (60%) subjek, ekspresi tropoelastin kuat pada 18 (60%) subjek dan ekpresi tropoelastin lemah pada 12 (40%) subjek. Dilakukan uji korelasi non parametrik dan didapatkan korelasi positif dengan nilai p=0,014 dan r=0,444. Derajat korelasi sedang.*

***Kesimpulan**: Terdapat korelasi positif antara ekspresi TGF-β1 dengan ekspresi tropoelastin pada ligamentum sakrouterina pasien dengan prolaps uteri dengan derajat korelasi sedang.*

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***Kata kunci**: prolapsus uteri, Transforming Growth Factor Beta 1, tropoelastin*

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INTRODUCTION

Uterine prolapse is the descent of the uterus from its normal position in the pelvic cavity into the vaginal canal or even out of the vagina. It is caused by the weakness of the muscles, fascia, and supporting ligaments.[1]

According to *Badan Pusat Statistik* (BPS), the life expectancy of Indonesian women will increase from 66.8 years in 2010-2015 to 72.2 years in 2030-2035. The longer life expectancy is, the more effort to improve the quality of life. Therefore, it is necessary to change the passive treatment paradigm of the pelvic organ prolapse (POP) to active preventive treatment.[1-3]

The prevalence of uterine prolapse was varied. In 2002, the Women's Health Initiative (WHI) reported the prevalence of uterine prolapse was 14% in women 50-79 years old; meanwhile, study in Dr. Hasan Sadikin hospital in 2006 found there were 30/1,455 cases (2.1%) of uterine prolapse and 13 of them was treated by vaginal hysterectomy. Fauzi A and Anhar K reported 43 cases of uterine prolapse in Dr. Mohammad Hoesin hospital Palembang during 1999-2003.[3-6]

Several risk factors of uterine prolapse are age, occupation, weight, parity, type of delivery, vaginal delivery using a vacuum or forceps, birth weight, surgical history, history of medical illness and

menopausal status. Actually, all of the risk factors may cause damage to the basic pelvic connective tissue, especially the cardinal and sacrouterine ligament.[6,7]

Sacrouterine ligament supporting and maintaining the uterus makes it in the appropriate position of the pelvic. Fixation function of the uterus is essential to prevent the further POP. Sacrouterine ligament is the first level in the pelvic support system according to Delancey. Sacrouterine ligament is composed of cells, extra cellular matrix consisting of fibers (collagen, elastin and reticulin), proteoglycans and glycoproteins. Abnormalities of the connective tissue of the pelvic floor and vaginal seem to have an important role in the pathophysiology of basic pelvic disorders.[7-11]

Transforming Growth Factor Beta 1 (TGF-β1) has a key role in the regulation of the extra cellular matrix and enzymes component. This TGF-β1 increases elastin matrix regeneration in vascular smooth muscle cells and a layer of skin fibroblasts.[1] Elastin is a protein in the extracellular matrix that affects the tension and elasticity of the tissue. Elastin plays an important role in maintaining the pelvic organs in order to be in the normal position. Women with genetic metabolic disorders, such as cutis Laxa, showed an increased risk for the occurrence of POP.[11-16]

Takacs P in 2011 reported a positive correlation between the expression of TGF-β1 and mRNA expression of elastin in the tissues of the vagina without pelvic floor disorders. In vitro, TGF-β1 is a potential regulator of the elastin production from the extra cellular matrix not only in pathological states, but also in the normal condition.[14] Previous studies revealed that the TGF-β1 increased the production of elastin in fibroblasts and vascular smooth muscle cells via mRNA elastin stabilization.[17-19] In addition, a decrease of the expression of elastin found in fibroblasts was derived from cardinal ligaments in POP women.[1]

Based on background above, this study aims to determine the correlation of TGF-β1 expression and tropoelastin expression of the sacrouterine ligament in women with uterine prolapse.

METHODS

The cross sectional study was carried out at the Department of Obstetrics and Gynecology Dr. Mohammad Hoesin hospital Palembang. The study was performed from December 1st, 2014 to July 31st, 2015 whereas only 30 subjects met the inclusion criteria.

The inclusion criteria were uterine prolapse women who were willing to sign an informed consent; while, the exclusion criteria were patients with connective tissue disorders or were undergoing the estrogen replacement therapy. They were doing the gynecological examination to determine the stage of uterine prolapse based on Pelvic Organ Prolapse Questionnaire (POP-Q) system.

We took the sacrouterine ligament in distal portion along 1 cm through total vaginal hysterectomy. We fixed the ligament into 10% buffered formalin, did the paraffin blocks manufacture, hematoxylin and eosin staining and also immunohistochemical examination to assess the expression of TGF-β1 and tropoelastin. Examination of TGF-β1 expression used monoclonal antibodies ab66403 and optimization of 1:75 was performed as the control. For the expression of tropoelastin, we used the monoclonal antibodies ab21598 with optimization of 1: 100. Mixture was read by a light microscope (Olympus BX 51 brands) with 400 times magnification and wide field of view 0.65 mm. The expression of TGF-β1 and tropoelastin were assessed by using a score based on the proportion of smeared cells and the intensity of light brown staining to dark brown. The interpretation was positive 1 (weak) if the smeared brown/vaguely into the cytoplasm extra cellular matrix ≤ 10% and positive 2 (strong) if the smeared dark brown/ darkness of the cytoplasm and the extra cellular matrix of >10%.

RESULTS

Table 1. Subject Characteristics

Characteristics	Number	Percentage
Age (years old)		
< 45	0	0
46- 55	10	33.3
56- 65	15	50.0
66- 75	4	13.3
76-85	1	3.3
Parity		
0	0	0
1-3	10	33.3
>3	20	66.7

Menopausal status		
Premenopause	1	3.3
Menopause	29	96.7
Type of delivery		
Never	0	0
Spontaneous	30	100.0
vacuum/Forceps	0	0
Sectio caesarean	0	0
History of giant baby		
Not known	11	36.7
< 3500 g	7	23.3
>3500 g	12	40
Body Mass Index		
Normal	16	53.3
Over weight	14	46.7
Degree of uterine prolapse		
II	6	20.0
III	15	50.0
IV	9	30.0

Table 2. Description of TGF-β1 and Tropoelastin Expression on Sacrouterina Ligament

No. Subject	Expression of TGF-β1		Expression of Tropoelastin	
	Strong	**Weak**	**Strong**	**Weak**
1.		+1		+1
2.		+1		+1
3.		+1		+1
4.		+1		+1
5.		+1		+1
6.	+2		+2	
7.		+1		+1
8.	+2		+2	
9.		+1	+2	
10.	+2			+1
11.		+1	+2	
12.		+1	+2	
13.		+1		+1
14.		+1		+1
15.		+1		+1
16.	+2		+2	
17.		+1		+1
18.	+2		+2	
19.	+2			+1
20.	+2			+1
21.		+1		+1
22.		+1		+1
23.		+1	+2	
24.		+1		+1
25.	+2			+1
26.	+2		+2	
27.		+1		+1
28.	+2		+2	
29.	+2		+2	
30.	+2		+2	

Table 3. Correlation of TGF-β1 and Tropoelastin Expression on Sacrouterine Ligament.

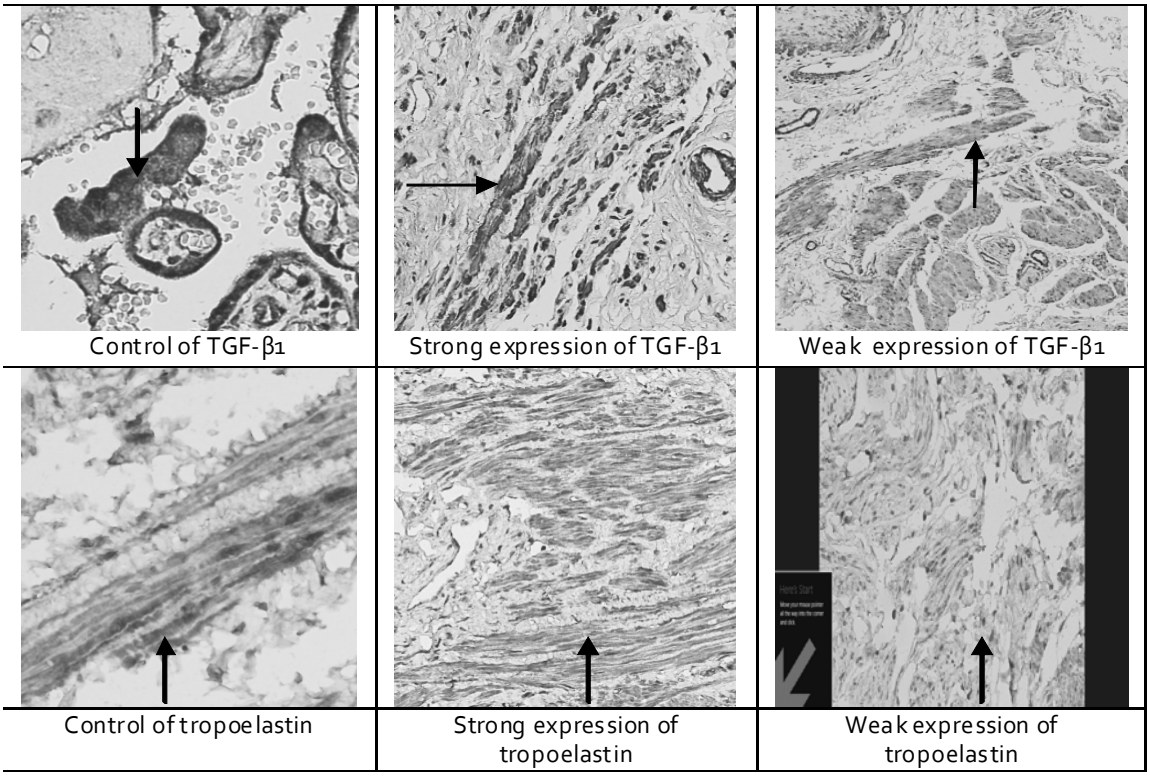
		Tropoelastin expression	
		Strong (%)	**Weak (%)**
TGF-β1	Strong	8 (66.7)	4 (33.3)
expression	Weak	4 (22.2)	14 (77.8)
Total		12 (40.0)	18 (60.0)

The subject characteristics, description of TGF-β1 and tropoelastin expression on sacrouterine ligament, and the correlation between both of them were shown in Table 1, 2 and 3; respectively. The statistical test result showed there was a relationship between the expression of TGF-β1 and tropoelastin (p=0.024). Non-parametric test performed and found that there was a positive correlation between the expression of TGF-β1 and tropoelastin in sacrouterine ligament with moderate correlation (p=0.014; r=0.44).

DISCUSSION

The imbalance between the synthesis and degradation of matrix extracellular components can cause POP.[20] In our study, we found there were 6 samples obtained the different expression between TGF-β1 and tropoelastin; it indicated the existence of another cytokine that induced the expression of tropoelastin.[20]

Cytokines which can regulate the components of the extracellular matrix are TGF-β1, thrombospondin I (TSP-I), matrix metalloprotease (MMPs), insulin growth factor (IGF-I), basic fibroblast growth factor (bFGF), Heparin binding Epidermal Growth Factor (Hb-EGF), Epidermal Growth Factor (EGF), Transforming Growth Factor Alpha (TGF-α), Tu-



mor Necrosis Factor Alpha (TNF-α). All of these cytokines may be involved in the regulation of elastin.[20-22] Further studies should be conducted to find the role of cytokines in the regulation of extra cellular matrix, especially on the tropoelastin components.

The correlation between the expression of TGF-β1 and tropoelastin did not explain the causal relationship of the two components. The weakness of our study was that we did not do the comparative assay of the TGF-β1 and tropoelastin expression in sacrouterine ligament from patients without uterine prolapse.

CONCLUSION

There is a positive correlation between the TGF-β1 and tropoelastin expression of sacrouterine ligament in uterine prolapse with moderate correlation.

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