ORIGINAL RESEARCH

Collagen-1 and elastin expression in cervical tissue: A comparison across cervical elongation, pelvic organ prolapse, and combined conditions

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ABSTRACT

Objective: This study aimed to assess differences in the expression of Collagen-1 and Elastin in cervical tissues among patients with Cervical Elongation (CE), Pelvic Organ Prolapse (POP), a combination of CE with POP, and those without either condition.

Materials and Methods: An analytical study with a cross-sectional design was conducted, using immunohistochemistry (IHC) to analyze cervical tissue samples preserved in paraffin blocks. Patient groups included those diagnosed with CE, POP, CE combined with POP, and a control group without CE or POP. All participants underwent surgery between January 2021 and April 2023. IHC was used to measure the expression levels of Collagen-1 and Elastin in each tissue sample. Observations were made under 400x magnification, focusing on five randomly selected visual-field areas in each sample to determine the area fraction. Two experienced pathologists conducted the analyses in a blinded manner to ensure objective evaluation.

Results: Statistical analysis using the Kruskal-Wallis test revealed significant differences in the expression of Collagen-1 across the four groups (CE, POP, CE with POP, and control). Patients with CE showed a higher expression of Collagen-1 than those with CE and POP combined, as well as the control group. However, no significant differences in Elastin expression were observed among the groups.

Conclusion: Collagen-1 expression differs significantly across patients with CE, POP, and CE combined with POP, suggesting a distinct role in cervical tissue remodeling in these conditions. Conversely, Elastin expression was consistent across all groups, indicating that it may not play a differentiating role in these pathologies. These findings highlight Collagen-1's potential involvement in the structural changes associated with CE and POP.

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Highlights:

1. Pathogenesis of cervical elongation is still limited, including the histological and molecular differences between a cervical elongation and a normal cervix.
2. The expression of collagen-1 level in the cervical elongation group was stronger compared to the cervical elongation with POP and control group.

INTRODUCTION

Cervical elongation is the lengthening or hypertrophy of the cervix towards the vaginal introitus with the supporting tissues of the uterus still in good condition.[1] Cervical elongation can occur in both parts of the cervix, the supravaginal part and the vaginal part. Supravaginal CE is found in 18% of patients with POP, while vaginal cervical elongation almost always occurs congenitally.[2] The cervix without adequate fixation from the sacrouterine ligament and pressure from the opposite direction by the pelvic floor muscles could result in cervical elongation. It is supported by the finding of a larger genital hiatus size in the group with CE compared to the POP patients without CE.[3]

The supporting structure of the female pelvic can be divided into three levels. Level 1 is the cardinal-uterosacral ligament complex; levels 2 and 3 consist of the fascia, urogenital diaphragm, and perineal bodies supporting the middle and lower parts of the vagina. Among the three grades, defects in grade 1 result in uterine prolapse. The POP condition is not life-threatening but causes a decrease in women's quality of life.[4] This condition is a common gynecological disorder, with approximately 37% of patients seeking treatment and the risk of surgical procedures being approximately 11-19% of the population.[5] The prevalence of POP in low-income countries such as Tanzania, Ethiopia, and Gambia is 46-64.6%.[6] In Indonesia, in 2007, there were reported 30 cases of grade III-IV prolapse, and there were around 20 operations on cases of grade III-IV uterine prolapse every year.[7] The prevalence of POP was 26.4%, urinary incontinence 15.3%, and fecal incontinence 2.5%.[8]

Not all patients with uterine prolapse experience reduction in the uterine corpus, as occurs in CE. In patients with CE, the supporting structures of level I remain relatively strong compared to uterine descent.[9] Approximately 40% of women with apical component prolapse experience CE, with the age of patients suffering from CE being younger than those suffering from POP. Women with POP were found to have a significantly higher ratio of cervical length to total uterine length when compared with women without POP.[10,11] Knowledge regarding the pathogenesis of CE is still limited, including the histological and molecular differences between a CE and a normal cervix. Most studies carry out examinations to determine the composition of supporting tissues such as the sacrouterine ligament and vagina, but there are still few for cervical tissue. Cervical elongation may have a different pathogenesis, not as a result of weak support of the uterosacral and vaginal ligaments, as has been reported in POP. It is suspected that CE is more of a local process than a systemic disorder, supported by their research, which found that the cervical tissue of patients with CE has higher levels of estrogen and progesterone receptors compared to normal cervix without elongation. The explanation for higher estrogen and progesterone receptor levels is a feedback effect of receptor regulation in response to reduced estrogen and progesterone content in these tissues.[10,12]

Collagen and elastin are important components of the extracellular matrix of cervical tissue. Type 1 collagen is the most abundant form (70%), and type 3 forms 30% of the total collagen. Collagen is an important component of the extracellular matrix that contributes to the biomechanical strength of the cervix. Elastin provides elasticity and stretchability, making up about 1.5% of the cervix of non-pregnant women. Smooth muscle and fibroblasts are cellular parts of the cervix.[13,14] From this study, researchers were interested in knowing the expression of collagen I and elastin in cervical tissue in CE group, POP group, CE with POP group, and group without CE or POP as a control.

MATERIALS AND METHODS

This is an analytical study with a cross-sectional approach. The study participants were cervical tissue patients' specimens from CE, POP, CE with POP, and without CE or POP who had underwent surgery at Dr. Soetomo Hospital during the research period and met the inclusion and exclusion criteria. Samples were determined by consecutive sampling technique. Inclusion criteria include cervical tissue patients' specimens from CE, POP-Q's grade 3 and 4 POP and CE with POP, and normal cervical tissue (without CE and POP). The exclusion criteria include a history of pelvic radiotherapy, hormone replacement therapy, a cervix exhibiting additional pathological characteristics (e.g., congenital abnormalities or precancerous lesions), and incomplete information medical records.

Diagnosis of CE, the length of the cervix and uterine corpus in the specimen are measured in centimeters. Cervical length > 3.38cm or the ratio of the cervix to the uterine corpus > 0.79 is considered as CE. Grade 3 and 4 POP is diagnosed with POP-Q system by measuring the position of the cervix when the patient in valsalva maneuver, and if the cervix descends beyond the hymen as far as between (+1 and TVL-2) then the subject is diagnosed with grade 3 POP and if > (TVL-2), subjects were diagnosed with grade 4 POP.

The Immunohistochemical (IHC) staining was used to determine the expression of Collagen-1 and Elastin level. The expression of Collagen-1 and Elastin in each

sample was viewed using a magnification of 400 times in 5 areas of the visual field were randomly selected to calculate the area fraction.[15] Reading of IHC results was performed by two experienced pathologists, both blinded to the clinical diagnosis of each sample. The collected data was tested for normality using the Shapiro-Wilk test. If it is normally distributed ($p > 0.05$) a Kruskal-Wallis test will be carried out to compare the levels of the dependent variable between the 4 groups. If the distribution is abnormal ($p < 0.05$), a non-parametric test will be carried out with the Mann-Whitney test. Statistical calculations using SPSS 26 software (IBM, Armonk, NY, USA). An ethical clearance letter with number 0653/KEPK/IV/2023 was received from Dr. Soetomo General Academic Hospital on April 17, 2023.

RESULTS AND DISCUSSION

Characteristics of research subjects

This research was carried out from May to September 2023. It started with collecting samples, then continuing with IHC examination at the Anatomical Pathology Laboratory, Dr. Soetomo General Academic Hospital, Surabaya, Indonesia. Samples were obtained from patient data registered from January 2021-April 2023. The total number of samples was 34 with 8 samples in group 1 (CE), 8 samples in group 2 (CE with POP), 9 samples in group 3 (POP), and 9 samples in group 4 (control). In the four groups, there were variations in subject age, number of parities, menopausal status and other conditions that were risk factors. If the medical record data is incomplete, we contact the subject by phone.

The mean age of the subjects was 51.94 ± 10.02. Meanwhile, for the number of parities, the average was 2.91 ± 1.68. Most of the subjects had a body mass index with overweight status. Most of the subjects did not experience constipation, long-term coughing and no history of pelvic organ surgery. The number of subjects with a history of weight lifting and those without was almost the same between 4 groups.

From statistical tests on the four groups, a significant difference in the mean age of the subjects was obtained ($p < 0.05$). The elongation group had the youngest mean age compared to the other three groups, namely 41.38 ± 3.93. Meanwhile, the POP group had the oldest mean age, 61.33 ± 5.34 (Table 1). These results are in accordance with several previous studies, where POP occurs in almost half of women aged over 50 years with a prevalence reaching 30-50%. POP cases at Dr. Soetomo General Academic Hospital in 2007-2011 showed that the average age of patients was 58.5±10.5 years. Approximately 40% of women with apical component prolapse experience CE, with the age of patients suffering from CE being younger than those suffering from POP.[11]

Table 1. Comparison of the proportion of cervical elongation, cervical elongation with POP, and POP based on patient's characteristics

	Groups				Total	p-values
	CE (n=8)	CE with POP (n=8)	POP (n=9)	Control (n=9)		
Age	8	8	9	9	34	
Mean ± SD	41.38±3.93	59.50±6.70	61.33±5.34	45.22±3.73		0.001
Menopause						
Yes (n%)	0 (0.00)	8 (44.44)	9 (50.00)	1 (5.56)	18	0.775
No (n%)	8 (50.00)	0 (0.00)	0 (0.00)	8 (50.00)	16	
Parities	8	8	9	9	34	0.007
Median (Min – max)	2.5(1 – 3)	3.5(2 – 6)	3 (2 – 9)	2 (0 – 3)		
Baby Birth Weight						
> 3500g (n%)	3 (25.00)	4 (33.33)	4 (33.33)	1 (8.33)	12	0.241
≤ 3500g (n%)	5 (22.73)	4 (18.18)	5 (22.73)	8 (36.36)	22	
Body Mass Index (BMI)						
Obesity (n%)	0 (0.00)	0 (0.00)	1 (100.00)	0 (0.00)	1	
Overweight (n%)	5 (2.73)	7 (31.82)	5 (2.73)	5 (2.73)	22	0.679
Normal (n%)	3 (27.27)	1 (9.09)	3 (27.27)	4 (36.36)	11	
Underweight (n%)	-	-	-	-		

There were no significant differences between the menopausal status of the four groups ($p > 0.05$). However, there were significant differences between the group of CE, CE with POP and the POP. All subjects in the CE group were not yet menopausal, whereas in CE with POP and POP groups, all were menopaused. In accordance with Ibeanu's research,12 the results of research on POP with CE, menopause is not a risk factor. Menopause is the main risk factor for POP, which is associated with a decrease of estrogen level which results in the genital tract atrophy, weakening of the pelvic floor muscles, uterosacral and cardinal ligaments, and a decrease in the ability of the endopelvic fascia to stretch.[7,17]

A case report suggests that a cervix without adequate fixation of the sacrouterine ligament and without pressure from the opposite direction by the pelvic floor muscles can lead to CE. This is supported by the finding of a larger genital hiatus size in the group with CE when compared to the group of POP patients without CE.[18] The symptoms of CE and apical POP that sufferers about are almost the same complain, but it is not yet clear whether CE and POP are different conditions or whether the two conditions always coexist. A study comparing women with and without POP, measuring the uterine corpus and cervix using MRI concluded that the cervix in women with uterine prolapse was 36.4% longer than in women without uterine prolapse ($p < 0.001$).[19,20]

There was a significant difference ($p < 0.05$) in parity in the four subject groups. The lowest average number of parities was owned by the control group (2 (0-3)) and the highest average number of parities was owned by the elongation group with POP (3.5(2-6)). Research by Liu11 provided significantly different results in parity between the elongation group and the POP group. Analysis of birth weight of babies born and BMI showed that there were no significant differences in the four groups ($p > 0.05$). This is in accordance with research by Liu which found that there was no significant difference in BMI between the elongation group and the POP group. [11

Expression of collagen-1 and elastin

After obtaining the data for the research samples in the four groups, a search was carried out for paraffin blocks of cervical tissue in the Anatomical Pathology Laboratory at Dr. Soetomo Hospital. Slides were made and an IHC was carried out for Collagen-1 and Elastin using the indirect immunoenzyme method. The examination used a Collagen-1 antibody kit (ABCLONAL/COL1A1 Rabbit pAb) and an Elastin antibody kit (ABCLONAL/ELN Rabbit pAb) with a dilution of 1:50.

In Figure 1 we can see the results of the CPI examination, viewed with an Olympus cx-31 microscope. Collagen-1 was stained positively in the cytoplasm of collagen fibers with strong intensity at microscopic magnifications of 200 times and 400 times. Measurement of Collagen-1 expression level on each slide was carried out in 5 random fields of view with 400x magnification.

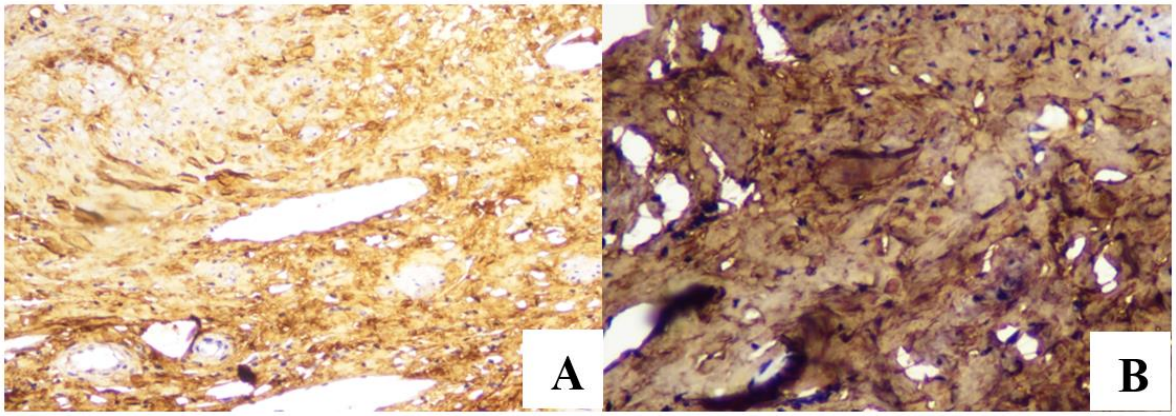

Figure 1. The results of IHC staining with collagen 1 antibodies showed positive staining in the cytoplasm of collagen fibers with strong intensity: A. 200x microscopic magnification, B. 400x microscopic magnification

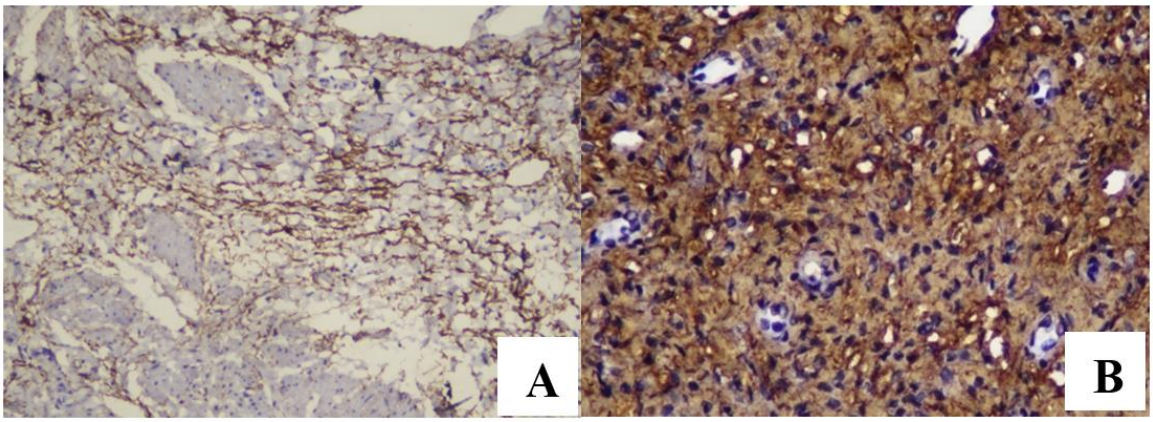

Figure 2. Results of IH staining with elastin antibodies on the cytoplasm of elastin fibers with strong intensity: A. 200x microscopic magnification, B. 400x microscopic magnification.

Table 2. Comparison of collagen-1 and elastin expression between the elongation group, elongation group with POP, POP group, and control group

	Groups				Total	p-values
	CE (n=8)	CE with POP (n=8)	POP (n=9)	Control (n=9)		
Collagen-1 expression						
Negative	-	-	-	-		
Low	-	-	-	-		
Moderate (n%)	0 (0.00)	2 (15.38)	7 (53.85)	4 (30.77)	13	0.010
High (n%)	8 (38.10)	6 (28.57)	2 (9.52)	5 (23.81)	21	
Elastin expression						
Negative(n%)	2 (25.00)	3 (37.50)	2 (25.00)	1 (12.50)	8	
Low (n%)	5 (35.71)	2 (14.29)	2 (14.29)	5 (35.71)	14	0.250
Moderate (n%)	1 (8.33	3 (25.00)	5 (41.67)	3 (25.00)	12	
High (n%)	-	-	-	-	-	

In Figure 2 we can see from the CPI results, Elastin appears to be colored brown. Elastin stained positively in the cytoplasm of Elastin fibers with strong intensity at microscopic magnifications of 200 times and 400 times. Measurement of Elastin expression on each slide was carried out in 5 random fields of view with a magnification of 400 times.

There was a significant difference in collagen-1 expression between the CE, CE with POP, POP, and control group with the results of the Kruskal-Wallis test p = 0.01 (Table 2). There was a significant difference in the proportion of subjects in the 4 study groups between subjects with moderate and strong Collagen-1 expression ($p < 0.05$). Most subjects with moderate Collagen-1 expression (n=7; 53.85%) were subjects with POP. Meanwhile, among 21 subjects with strong collagen 1 expression, 38.10% (n=8) were subjects with elongation and only 9.52% (n=2) were subjects with POP. This shows that the POP group is the group with the most subjects who have lower collagen 1 expression compared to the other 3 groups and the fewest number of subjects who have strong collagen expression. On the other hand, the group with elongation had the highest number of subjects with strong Collagen-1 expression compared to the other 3 groups, and the fewest number of subjects had moderate Collagen-1 expression.

Table 3. Comparison of collagen-1 expression between groups

Groups	CE	CE + POP	POP
CE + POP	1.000	-	-
POP	0.012	0.239	-
Control	0.025	1.000	1.000

The Mann-Whitney post hoc test resulted in a significant difference in Collagen-1 expression in the comparison between CE and POP group (p = 0.012) and between CE and control group (p = 0.025) (Table 3). The number of samples with strong Collagen-1 expression level in CE was greater (n=8; 61.54%)

compared to POP (n=2; 22.2%) and control group (n=5; 38.46%), respectively. Comparison of Collagen-1 expression between other groups did not reveal any significant differences. The results of the Kruskal-Wallis test showed no significant differences in Elastin expression levels between CE, CE with POP, POP group and control group. There was a significant difference in the proportion of subjects in the 4 research groups between subjects with negative, low and moderate elastin expression ($p < 0.05$). The group with overall moderate elastin expression (n = 9) was the control group. Meanwhile, of the 18 subjects with strong elastin expression, 50% (n = 9) were the control group and only 5.56% (n = 1) were the group with elongation. Despite the overall results, this study certainly had limitations. First, samples were surgical specimens from the last 3 years, there is a possibility of changes in tissue properties. Second, there is possibility any bias in diagnosing CE because it is not done by one person.

CONCLUSION

The expression of Collagen-1 level in the CE group was stronger than in control group and CE with POP group, while the expression of Elastin in the CE, CE with POP, and POP did not have a significant differ from control group.

DISCLOSURES

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Conflict of interest

We, all authors have no conflict of interest.

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Author contribution

E Mardiyan Kurniawati, G Hardianto project development, manuscript editing, and approval of the final manuscript. E Hari Kusumastuti, B Iman Santoso, and B Utomo manuscript editing and approval of the final manuscript. A Widyasari project development, data management and analysis, manuscript editing, and approval of the final manuscript.

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