ORIGINAL ARTICLE

Comparison of pain intensity, smooth muscle cells density, and α-smooth muscle actin expression in ovarian and peritoneal endometriosis

Sutrisno*, Muhammad Nooryanto, Shella Widya Gani
Department of Obstetric and Gynecology, Faculty of Medicine, Brawijaya University, dr. Saiful Anwar Hospital, Malang, Indonesia

ABSTRACT

Objectives: to identify the role of smooth muscle through the analysis of smooth muscle cells density, expression of α-SMA, and pain intensity.
Materials and Methods: The study design was cross-sectional analytic observational. Study sample consisted of women with ovarian endometrios and women with peritoneal endometriosis who underwent laparoscopy and laparotomy in Dr. Saiful Anwar Hospital and RSIA Melati Hospital, both in Malang, Indonesia, from January until December 2019. There were 16 samples: 8 samples of ovarian endometriosis and 8 samples of peritoneal endometriosis. Smooth muscle cell density was analyzed by comparing the number of smooth muscle cells with the total area of endometriosis tissue in one microscopical field. α-SMA expression was obtained by immunohistochemistry. The degree of pain was determined by filling the part 1 point 1-11 of EHP-30 queistionnaire the day after the procedure. Data were analyzed with Independent T-test and Pearson's correlation test.
Results: Pain intensity, smooth muscle cells density, and α-SMA expression was higher in endometriosis patients compared to healthy individuals. Pain intensity, smooth muscle cells density, and α-SMA expression was lower in the ovarian endometriosis compared to peritoneal endometriosis.
Conclusion: There was a significant correlation between the expression of α-SMA, smooth muscle density, and pain intensity in endometriosis.

Keywords: Ovarian endometriosis; peritoneal endometriosis; α-SMA expression; smooth muscle cells density; pain intensity

ABSTRAK

Tujuan: mengetahui peran otot polos melalui analisis densitas otot polos and ekspresi α-SMA terhadap derajat nyeri.
Bahan dan Metode: Studi ini merupakan studi observasional cross sectional analytic. Sampel adalah wanita dengan kista endometriosis dan wanita dengan peritoneal endometriosis yang menjalani laparoskopi dan laparotomi di RSUD Saiful Anwar dan RSIA Melati, Malang, Indonesia, pada bulan Januari – Desember 2019. Didapatkan 16 sampel dengan 8 sampel berasal dari pasien kista endometriosis dan 8 lainnya berasal dari pasien peritoneal endometriosis. Densitas otot polos dianalisis dengan membandingkan jumlah otot polos dengan total area jaringan endometriosis pada satu lapang pandang. Ekspresi α-SMA dianalisis menggunakan imunohistokimia. Derajat nyeri didapatkan menggunakan kuisioner EHP-30 bagian 1 poin 1-11 satu hari setelah prosedur. Analisis data menggunakan Uji T independen dan korelasi Pearson.
Hasil: Derajat nyeri; densitas otot polos, dan ekspresi α-SMA yang lebih tinggi pada pasien endometriosis dibandingkan dengan individu sehat. Derajat nyeri, densitas otot polos, dan ekspresi α-SMA didapatkan lebih rendah pada kista endometriosis dibandingkan dengan peritoneal endometriosis.
Simpulan: Terdapat korelasi yang signifikan dari ekspresi α-SMA, densitas otot polos dengan derajat nyeri pada endometriosis

Kata kunci: Kista endometriosis; peritoneal endometriosis; ekspresi α-SMA; densitas otot polos; derajat nyeri

***Correspondence:** Sutrisno. Department of Obstetric and Gynecology, Faculty of Medicine, Brawijaya University, Malang, Indonesia/dr. Saiful Anwar Hospital, Malang, Indonesia. E-mail: snospogk@gmail.com

● pISSN:0854-0381 ● eISSN: 2598-1013 ● doi: http://dx.doi.org/10.20473/mog.V29I32021.108-117
● Maj Obs Gin. 2021;29:108-117 ● Received 14 May 2021 ● Revised 30 Jul 2021 ● Accepted 13 Aug 2021

INTRODUCTION

Endometriosis is a gynecological pathology when there is an implantation of abnormal tissues that resemble endometrium outside the uterus that induce chronic inflammation.[1] Implantation most often found in ovarium, tuba fallopii, bladder, rectosigmoid colon, and myometrium of the uterus. Endometriosis known as the cause of infertility that stil could not be resolved until now.[2]

Endometriosis found more often in women with reproductive age. A study conducted by Surrey et al found that the mean age of endometriosis case in American is 36 years.[2] The prevalence of endometriosis in fertile women is 0,5-5% while in unfertile women is 25-40%.[3]

The pathogenesis of endometriosis is still controversial however there are 3 widely accepted theories which are retrograde menstruation, coelomic metaplasia, and endometriosis induction. There are a distinct prevalence of retrograde menstruation and endometriosis. This indicate that there are many other factors that also related to the pathogenesis of endometriosis that include specific molecular abnormalities or defect in immunity.[4]

Pain, as one of the clinical finding in endometriosis, is a multifactorial manifestation. Inflammatory response known to differ pain.[4] Smooth muscles contraction in the endometriotic lesion could also produce pain by stimulating the nociceptor. Smooth muscles was found in all endometriosis type (ovarian, peritoneal, and deep infiltrating).[5]

Hyperplasia and hypertrophy of smooth muscles in endometriotic lesion consistently show expression of α-smooth muscle actin (α-SMA). Increase of the number and density of smooth muscles accompanied by contractility of the muscles known to be related to the induction of pain in endometriosis.[6]

Role of smooth muscles in the presentation of pain lead to an analysis to differentiate the density of smooth muscles also the expression of α-SMA in endometriotic lesion. The relation of smooth muscle and α-SMA expression with the pain intensity also being observed. This study was conducted in the Physiology Laboratory of Brawijaya Medical Faculty in the year of 2020.

MATERIALS AND METHODS

This study was conducted with cross sectional analytic observational design. The study sample consist of women with ovarian endometriosis and women with peritoneal endometriosis that undergo laparoscopy and laparotomy in RSUD Dr. Saiful Anwar, Malang, Indonesia and RSIA Melati Malang, Indonesia, within January until December of 2019. According to the formula of study sample with two independent variables, the sample number needed are 16 : 8 samples of ovarian endometriosis and 8 samples of peritoneal endometriosis.

The smooth muscles density in endometriosis tissue was done by making a comparisson of smooth muscles number with the total area of endometriosis tissue for each microscopical field with 400x magnification. The smooth muscle density with actin positive in 10 microscopical field also being calculated. α-SMA expression was identified by calculating the percentage of area that express the α-SMA using immunohisto-chemistry and the analysis was done by Image J software. The pain intensity was observed using Endometriosis Health Profile Questionnaire (EHP-30) part 1 point 1-11 one day after the procedure.

Inclusion and exclusion criteria

Inclusion criteria: women with endometriotic lesion (ovarian or peritoneal) age 18-49 years old, agreed to the procedure of laparoscopic and laparotomy, not having any hormonal therapy in the past 3 months. Exclusion criteria: women with endometriosis that also has degenerative problems, endocrine problems, contra-indicated to the procedure (bowel obstruction, ileus, peritonitis, intraperitoneal hemorrhage, and cardio-respiration problems), pregnant.

Data analysis

Data was analyzed using the SPSS. Normality test was done by Shapiro Wilk and Independent T test for the comparisonal study. The relation of two variables was analyzed by Pearson correlation.

RESULTS AND DISCUSSION

The sample characteristic of this study was shown on Table 1.

Tabel 1. Sample characteristics

Characteristic	Group (Mean ± SD)		p-value
	Ovarian (n = 8)	Peritoneal (n = 8)	
Age (years)	31.75 ± 3.15	36.63 ± 6.05	0.063

According to Table 1, the mean age of the ovarian endometriosis sample is 31.75 ± 3.15 years old and for

the peritoneal endometriosis is 36.63 ± 6.05 with p=0.063. P-value greater than 0.05 shows there are no significant difference in the age of the sample in both groups.

Data collected from normality test using Saphiro-Wilk, was shown on Table 2.

Table 2. Result of normality test

Variable	Saphiro-Wilk Test Result	
	Ovarian (n = 8)	Peritoneal (n = 8)
α-SMA Expression Smooth Muscle Cells Density	0.839 (p = 0.073)	0.973 (p = 0.917)
	0.908 (p = 0.340)	0.848 (p = 0.091)
Pain Intensity	0.869 (p = 0.146)	0.906 (p = 0.327)

Corresponding to the normality test result shown in Table 2, the comparative study of α-SMA expression, smooth muscle cells density, and pain intensity could be done using the independent T-test. According to observation done in one healthy individual, the pain intensity obtained is 1.82. The comparison of pain intensity in endometriosis patient and healthy individual was shown in Table 3.

Table 3. Comparison of pain intensity in endometriosis patient and healthy individual

Group	Mean ± SD	Healthy	p-value
Ovarian	31.84 ± 19.33	1.82	0.003
Peritoneal	59.38 ± 5.22		0.000

According to Table 3, pain intensity in ovarian endometriosis is 31.84 ± 19.33 while the intensity in peritoneal endometriosis is 59.38 ± 5.22. If we compare the result with pain intensity in the healthy individual (1.82), the p-value is less than 0.05 in both groups which indicate that there is a significant increase of pain intensity in the endometriosis patient compared to the healthy individuals.

Then the pain intensity in the ovarian and peritoneal endometriosis patient was compared using the Independent T-test. Figure 1 shows the comparison of pain intensity in the two groups of endometriosis that shows a lower pain intensity in ovarian endometriosis patient compared to the peritoneal endometriosis patient (p = 0.002).

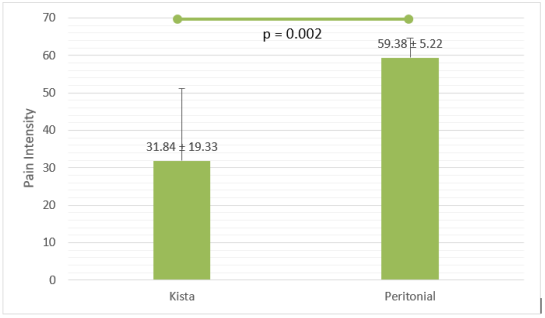

Figure 1. Pain intensity of ovarian endometriosis and peritoneal endometriosis

Observation of smooth muscle cells density in one healthy individual shows a result of 7,3. The comparison of smooth muscle cells density in the endometriosis patient and helathy individual was shown in Table 4.

Table 4. Comparison of smooth muscle cells density in endometriosis patient and healthy individual

Group	Mean ± SD	Healthy	p-value
Ovarian	44.05 ± 3.76	7.3	0.000
Peritoneal	50.01 ± 2.87		0.000

According to Table 4, the mean density of smooth musle cells in ovarian endometriosis is 44.05 ± 3,76 while in the peritoneal endometriosis is 50.01 ± 2.87. Comparing the result with healthy individual (7.3) we obtained p<0.05 that indicate there is a significant increase in the density of smooth muscle cells in endometriosis.

Then we compare the smooth muscle cells density in the two groups of endometriosis patient using Independent T-test. Figure 2 shows the compration of smooth muscle cells density in ovarian and peritoneal endometriosis. The result shows that the mean density in ovarian endometriosis is lower than the peritoneal endometriosis (p = 0.003).

Observation of α-SMA in one healthy shows an expression of 1.51%. The comparison of α-SMA expression in endometriosis patient and healthy individual was shown in Table 5. According to Table 5, the mean expression of α-SMA in the ovarian endometriosis is 20.49 ± 7.65% while in the peritoneal endometriosis is 64.19 ± 9.4%. If the result is being compared with the α-SMA expression in healthy individual (1.51%) we obtain p<0.05 that implivate the the increase of α-SMA expression in both endometriosis group is statistically significant.

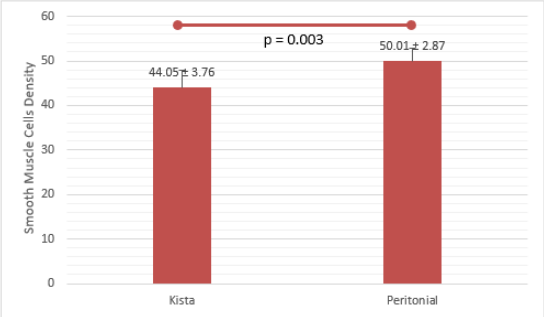

Figure 2. Smooth muscle cells density in ovarian endometriosis and peritoneal endometriosis.

Table 5. Comparison of α-SMA expression in endometriosis patient and healthy individual

.Group	Mean ± SD	Healthy	p-value
Ovarian	20.49 ± 7.65	1.51	0.000
Peritoneal	64.19 ± 9.4		0.000

In the aim of knowing the difference of α-SMA expression in the ovarian and peritoneal endometriosis, we did comparative study using the Independent T-test. Figure 3 shows the comparison of α-SMA expression in ovarian and peritoneal endometriosis. The mean expression of ovarian endometriosis is lower than the peritoneal endometriosis with p-value of 0,000.

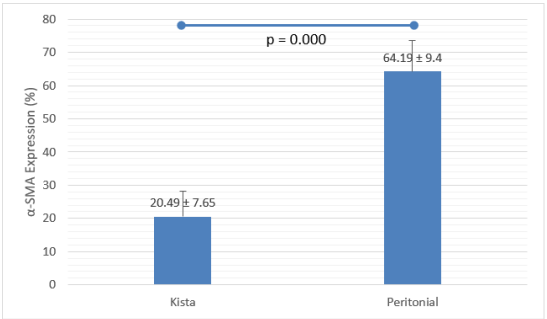

Figure 3. α-SMA expression in ovarian endometriosis and peritoneal endometriosis

To know the relation of α-SMA expression, smooth muscle cells density, and pain intensity, we did Pearson correlation test. The result was shown in Table 6. According to Table 6, the correlation coefficient of α-SMA expression and smooth muscle cells density is 0.640 with p=0.008. P-value less than 0.05 shows that there are a significant correlation between α-SMA expression and smooth muscle cells density. Correlation coefficient of 0.640 shows that there is a strong correlation. The positive number shows that the correlation between the variables is positive so in this case, when there is an increase in the expression of α-SMA, the smooth muscle cells density will also increase.

Table 6. Correlation test result of α-sma expression, smooth musle cells density, and pain intensity

Relation	Correlation	p-value	Implication
α-SMA Expression with Smooth Muscle Cells Density	0.640	0.008	Significant
α-SMA Expression with Pain Intensity	0.646	0.007	Significant
Smooth Muscle Cells Density with Pain Intensity	0.472	0.065	Not Significant

In the correlation test between α-SMA expression and the pain intensity in Table 6, the correlation coeficient is 0.646 with p=0.007. The result indicate that there are a strong correlation between the α-SMA expression and pain intensity.

The correlation between smooth muscle cells density and pain intensity (Table 6) shows correlation coefficient of 0.472 with p=0.065. P-value greater than 0.05 shows there are no significant correlation with smooth muscle cells density and pain intensity. The relation of α-SMA expression, smooth muscle cells density, and pain intensity in each group of endometriosis was also being conducted. The relation of all variables was analyze using Pearson correlation test. The result of correlation test in the ovarian endometriosis sample was shown in Table 7.

Table 7. The result of correlation test between α-SMA expression, smooth muscle cells density, and pain intensity in ovarian endometriosis sample

Relation	Correlation	p-value	Implication
α-SMA Expression and *Smooth Muscle Cells* Density	-0.280	0.502	Not Significant
α-SMA Expression and Pain Intensity	-0.264	0.527	Not Significant
Smooth Muscle Cells Density and Pain Intensity	-0.031	0.942	Not Significant

According to Table 7, in ovarian endometriosis sample the relation of α-SMA expression with the smooth muscle cells density has a correlation coefficient of -

0.280 with p-value of 0.502. P-value greater than 0.05 shows there are no significant correlation in the expression of α-SMA with smooth muscle cells density.

In the correlation test of α-SMA expression with pain intensity in the ovarian endometriosis, the correlation coefficient is -0.264 with p=0.527 (Table 7). P>0.05 indicate that there are no significant correlation between α-SMA and pain intensity.

The correlation test result of smooth muscle cells density and pain intensity (Table 7) shows a correlation coefficient of -0.031 with p=0.942. P>0.05 indicate that there are no significant correlation between smooth muscle cells density and pain intensity in the ovarian endometriosis sample.

The identical test was done with peritoneal endometriosis sample to identify the relation of α-SMA expression, smooth muscle cells density, and pain intensity. The correlation test result of the 3 variables in peritoneal endometriosis sample was shown in Table 8.

Table 8. The result of correlation test between α-SMA expression, smooth muscle cells density, and pain intensity in peritoneal endometriosis sample

Relation	Correlation	p-value	Implication
α-SMA Expression and *Smooth Muscle Cells* Density	0.238	0.571	Not Significant
α-SMA Expression and Pain Intensity	0.167	0.693	Not Significant
Smooth Muscle Cells Density and Pain Intensity	-0.165	0.696	Not Significant

According to Table 8, in the peritoneal endometriosis sample the relation between α-SMA expression and the smooth muscle cells density shows a correlation coefficient of 0.238 with p=0.571. P-value greater than 0.05 shows there are no significant correlation between the expression of α-SMA with the smooth muscle cells density.

In the correlation test result of the α-SMA expression with pain intensity in peritoneal endometriosis as shown in Table 8, the correlation coefficient is 0.167 with p=0.693. P>0.05 shows there are no significant correlation between the expression of α-SMA with pain intensity.

Correlation test between smooth muscle cells density and the pain intensity (result shown in Table 8), the correlation coefficient is -0.165 with p=0.696. P-valule greater than 0.05 shows there are no significant correlation between the smooth muscle cells density and the pain intensity in the peritoneal endometriosis sample.

Study sample characteristic analysis

Statistically, there are no significant difference in the age population between ovarian and peritoneal endometriosis so the characteristic between the two group is identical. Ashrafi (2016) showed a difference in the age of infertile women with and without endometriosis (32.4 ± 4.9 vs 31.4 ± 5.2; p=0.02).[7] In the study conducted by Khan (2012), from the total of 2988 laparoscopic procedure, the endometrioma cyst was found in women age 20-29 years old (31.4%) or 30-39 (51.7%). Both of the groups are still considered as premenopausal age.[8]

Analysis of pain intensity comparison in endometriosis patient

Analysis of pain intensity comparison in patient with endometriosis and healthy individual

In the last decade, the theory of a novel nerve fibers that induce the pain manifestation in endometriosis is widely accepted. Anaf (2000), found that in deep infiltrating endometriosis that has the highest pain intensity, the nerve fibers are denser in the location of endometriotic lesion. In the patient with more intense pain, endometriosis could invade the neuron (intraneural invasion) or the area adjacent to the nerve fiber (perineural invasion).[9,10]

Analysis of pain intensity comparison in patient with ovarian endometriosis and peritoneal endometriosis

Chiantera et al (2017) found that in endometriosis patient, the patient could feel visceral and somatic pain concomitantly and it depends on the location of the endometriosis. Visceral pain is triggered by the involvement of other organs (uterus, bladder, bowel). Meanwhile the somatic pain will be felt if the endometriosis located in the pelvic wall, muscle, or joint.[11]

Anatomically, peritoneum is a structure that is innervated by a bunch of nerve fibers. Unlike peritoneum, ovarium is relatively less sensitive to pain.[11] This founding lead to some hypothesis formulation that include: a. The pain intensity of endometriosis is related to the depth of the infiltration.[12] b. The pain intensity of endometriosis is affected by the anatomical location of the implantation. The endometriosis location could be stratified based on the intensity of the pain: uterosacral ligament, vagina, bladder, bowel, and urether (starting

from the mildest to the most intense pain).[12] c. In the case of severe endometriosis with ovarian cyst and deep infiltration, pain could be generated from the hip distortion and wide adhesion in the hip.[12] d. Factor contributing to pain generation is multifactorial, including: anatomical distortion of the hip, hip adhesion, cyclical bleeding inside the lesion, pelvic inflammation, pain generator substances in the peritoneal fluid, hip nerves irritation and infiltration, neurogenesis, and neuropathy.[12]

Analysis of smooth muscle cells density comparison in endometriosis patient

Analysis of smooth muscle cells density in patient with endometriosis and healthy individual

Widely accepted theory of endometriosis is the retrograde menstruation. Retrograde menstruation is a condition when there is an endometrial tissue reflux to the tuba fallopii. The tissue then implanted to the peritoneal surface or pelvic organ and develop according to the hormonal cycle. Other theory that is also widely accepted is the metaplastic theory where the peritoneal cell is differentiated into functional endometrial tissue. The induction theory is a combination of retrograde menstruation and metaplastic theory. Endometrium secreted a substance that differentiate the undifferentiated mesenchyme into endometrial tissue.[13]

Anaf et al (2000) discovered that ovarium, uterosacral, and rectovaginal endometrium significantly comprised of more smooth muscle cells compared to the unaffected location ($p<0.001$).[9] This finding is coherent with our study. In the normal ovarium, the smooth muscle cells that were stained by actin commonly located in the stromal while in the ovarian endometriosis the stained cells were located in the cortex. This finding proved the hypothesis that ovarian endometriosis was a product of ovarian mesothelium metaplasia that was invaginated to the ovarian cortex.

The result of hematoxilyn eosin staining of the ovarian and peritoneal endometriosis was shown in Figure 4. Our study shows a significant difference of smooth muscle cells density in endometriosis patient compared to the patient without endometriosis. Smooth muscle cells is more prevalent in the endometriotic lesion compared to the normal adjacent tissue or the eutopic endometrium. The smooth muscle cells found in black peritoneal lesion is denser than in red lesion and it shows the metaplastic phenomenon in the pathogenesis of endometriotic lesion.[9]

Analysis of smooth muscle cells density in patient with ovarian endometriosis and peritoneal endometriosis

Histologically, in deep endometriotic lesion the endometrium-like tissue is minimal. The main component of nodular lesion is not endometrial tissue but the fibromuscular tissue with extension to the gland and stroma.[14]

Zhang et al., stated that endometriotic lesion is basically a structure that undergo repetitive process of trauma and healing (ReTIAR) that eventually resulted in fibrosis because of thrombocyte aggregation.[15] A similar theory of thrombocyte activation stated that it promote the process of epithelial-to-mesenchymal transition/EMT, fibroblast-to-myofibroblast transition/FMT, and smooth muscle differentiation in the ovarian and deep infiltrating endometriosis.[16,17]

Metaplasia of the smooth muscle cells is a response of injury. Kim et al., stated that their case is the first smooth muscle nodular metaplasia in the peritoneal endometriosis. Factor provoking the transformation in the metaplastic smooth muscle remains unknown.[18]

Smooth muscle cells found in peritoneal endometriosis could be from the proliferation of smooth muscle cells in endometriotic foci or endometriosis stroma that undergo metaplasia to smooth muscle. Multipotent stem cell found in the endometrium stroma could also related to the generation of smooth muscle.[19]

A significant difference of smooth muscle cells in ovarian, uterosacral, and uterovaginal endometriosis remains unexplained. The infiltration rate was known to be dependent to the plasma concentration of sex steroid while in ovarian cyst there could be a possibility of relation between microenvironment and steroid concentration.[9]

Our study found that the density of smooth muscle cells in ovarian endometriosis is less than peritoneal endometriosis. Statistically, the difference in both group is significant.

Matsuzaki et al., that identify the EMT process using molecular marker in all form of endometriosis, found that the expression of epithelial marker (cytokeratin) is lower than mesenchymal marker (vimentin) in the red peritoneal lesion and ovarian endometriosis compared to menstrual endometrium. This finding shows that endometrium epithelial cell might undergo EMT process after the implantation of endometrium to the peritoneum, resulting in red lesion that is more invasive.[20] In contrast, the black peritoneal lesion and deep infiltrating endometriosis shows more expression of epithelial marker (E cadherin) compared to the menstrual endometrium and red lesion. This finding

indicate that there is an EMT-like process in the evolution of deep infiltrating endometriosis.[21,22]

Analysis of α-SMA expression in endometriosis patient

Analysis of α-SMA expression in endometriosis patient and healthy individual

Our study shows a significant increase of α-SMA expression in endometriosis patient. α-SMA is the main component of smooth muscle cells and the antibody anti α-SMA could be use in the detection of smooth muscle cells metaplasia due to its specificity to smooth muscle cells.[5] In the normal endometrial tissue, we can found staining of α-SMA but in fact it is the staining of myometrium and vascular smooth muscle.[23]

Zhang et al (2016) stated that platelet has a key role in the development of endometriosis because in fact endometriosis is a tissue that undergo repetitive trauma and healing process. Endometriosis was stimulated by platelet-derived transforming growth factor β1/TGF-β1 that lead to activation of TGF-β1/Smad3 signalling pathway and also EMT and FMT process. This resulted in increase of cellular contractility, collagen production, smooth muscle metaplasia/SMM, and eventually lead to fibrosis. As endometriosis progress, TGF-β1 and Smad3 increase and the number of α-SMA positive myofibroblast and differentiating smooth muscle cells in stroma is also increase. This finding is harmonious with our study finding.[16]

Analysis of α-SMA expression in ovarian endometriosis and peritoneal endometriosis

An experimental study in rat model shows that signalling pathway of STAT3 is a strong generator of EMT, FMT, and SMM in the epithelial and stromal endometriotic lesion that leads to increase of contractility, colagen deposit, and fibrosis.[24,25] Subcoelomic mesenchymal smooth muscle cell was identified in the biopsy of endometrial peritoneal lesion, mostly from the uterosacral ligament and the lateral wall of hip meanwhile biopsy result without smooth muscle mostly found in pararectal or rectal serous. Neovascularization was also observed.[26]

Analysis of the correlation between α-sma expression and smooth muscle cells density

α-SMA was found in the smooth muscle cells, pericyte, myoepithel, and normal endometrial stroma. In endometrium, α-SMA positive stromal cells (SMA-SC) and α-SMA expression was regulated by estrogen.[5]

Odagiri et al found that the interstitial in the endometrial lesion from human and rat model is denser than control. In the interstitial fibrotic area, positive immunostaining for α-SMA and Neural cell adhesion molecule (NCAM) was also found while there were none in the normal endometrium. Staining of inflammatoric cell by anti-NGF in the endometriotic interstitium was also found. This finding indicate that the main pathological process of endometriosis is the smooth muscle metaplasia and induction of nerve by the inflammatory cells like macrophage and lymphocyte.[23]

Analysis of the correlation between α-sma expression and pain intensity

Other mechanisms that could generate endometriotic pain includes cytokine release by macrophage that induce smooth muscle metaplasia and nerve fiber. Smooth muscle contraction and also hyperalgesia of sensoric fibers could also resulted in endometriotic lesion.[23]

Study conducted in other organs found that there are a relation between α-SMA expression and pain intensity due to the smooth muscle contraction. In the in vivo and in vitro study conducted by Alarcon-Martinez, α-SMA was found to have a role in brain and retinal capillary contraction by alternating between contraction and relaxation.[26]

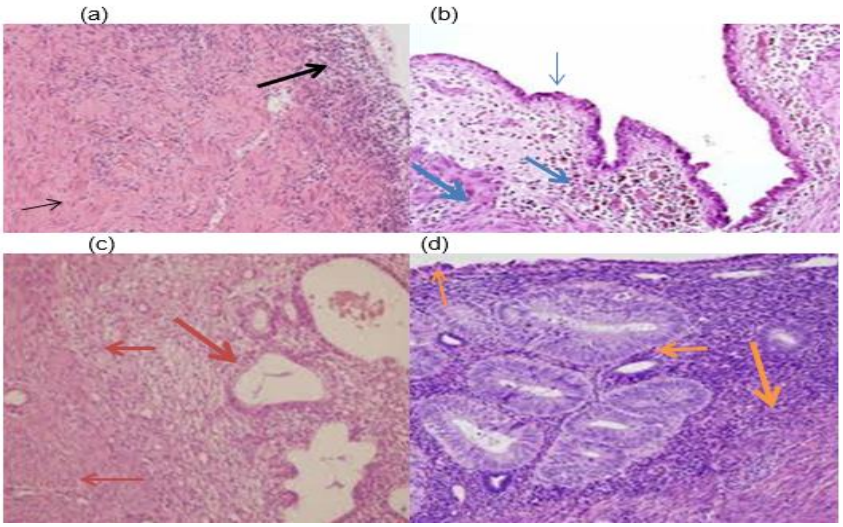

Figure 4. Hematoxylin Eosin staining in the negative control, peritoneal endometriosis, and ovarian endometriosis. (a) Peritoneum without endometriosis (thin black arrow: peritoneal fibrous stroma; thick black arrow: chronic inflamatory with lymphocyte in the margin) (b) Cyst without endometriosis (thin blue arrow: cuboid epithelial; medium blue arrow: loose connective tissue with hemosiderophage; thick blue arrow: stroma of fibrous tissue) (c) Peritoneal endometriosis (thin red arrow: peritoneal fibrous stroma; medium red arrow: smooth muscle cell; thick red arrow: gland with endometrial stroma (d) Ovarian endometriosis (thin orange arrow: cyst wall with cuboid epithelial; medium orange arrow: endometrial stroma and gland; thick orange arrow: smooth muscle cell). Using Hematoxylin Eosin in 100x magnification.

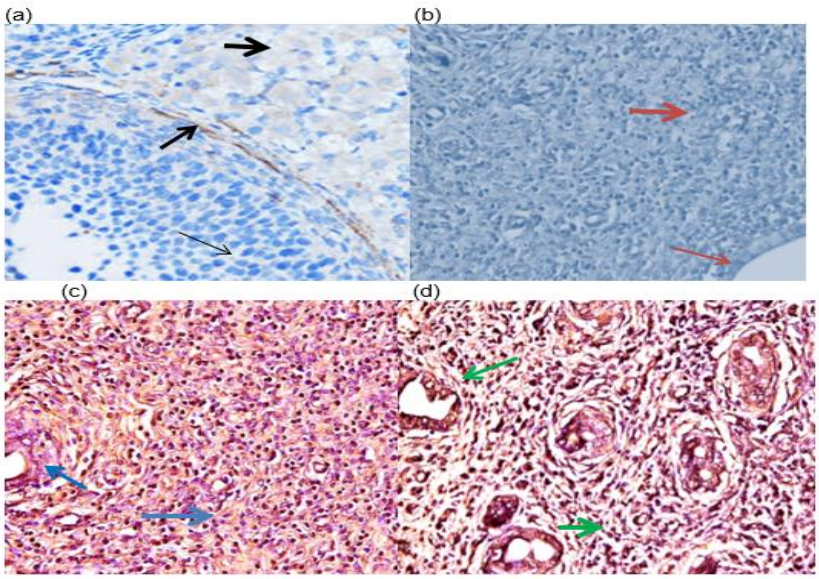

Figure 5. Immunohistochemistry of α-SMA in the negative control, peritoneal endometriosis, and ovarian endometriosis. (a) Peritoneum without endometriosis (thin black arrow: peritoneal stroma; medium black arrow: peritoneal visceral smooth muscle; thick black arrow: subperitoneal fat) (b) Cyst wall without endometrium (thin red arrow: cuboid epithelial; thick red arrow: stromal) (c) Peritoneal endometriosis (thin blue arrow: endometrial stroma and gland; thick blue arrow: smooth muscle cell (d) Ovarian endometriosis

CONCLUSION

This study is a relatively new area of research but have a critical clinical correlation. The mechanism of smooth muscle metaplasia development in endometriosis is still poorly understood and the research concerning this topic remains limited so it is a challenge to search for reference.

REFERENCES

1. Czyzyk A, Podfigurna A, Szeliga A, Meczekalski B. Update on endometriosis pathogenesis. Minerva Ginecol. 2017;69(5):447-461. doi: 10.23736/S0026-4784.17.04048-5. Epub 2017 Mar 7. PMID: 28271702.
2. Surrey ES, Soliman AM, Johnson SJ, et al. Risk of Developing Comorbidities Among Women with Endometriosis: A Retrospective Matched Cohort Study. J Womens Health (Larchmt). 2018;27(9):1114-1123. doi: 10.1089/jwh.2017.6432. Epub 2018 Aug 2. PMID: 30070938.
3. Ozkan S, Murk W, Arici A. Endometriosis and infertility: epidemiology and evidence-based treatments. Ann N Y Acad Sci. 2008;1127:92-100. doi: 10.1196/annals.1434.007. PMID: 18443335.
4. Eisenberg VH, Zolti M, Soriano D. Is there an association between autoimmunity and endometriosis? Autoimmun Rev. 2012;11(11):806-14. doi: 10.1016/j.autrev.2012.01.005. Epub 2012 Feb 4. PMID: 22330229.
5. Barcena de Arellano ML, Vercellino AJ, Chiantera V, et al. Mechsner S. Influence of nerve growth factor in endometriosis-associated symptoms. Reprod Sci. 2011;18(12):1202-10. doi: 10.1177/1933719111410711. Epub 2011 Jun 14. PMID: 21673280.
6. Koike N, Tsunemi T, Uekuri C, et al. Pathogenesis and malignant transformation of adenomyosis (review). Oncol Rep. 2013;29(3):861-7. doi: 10.3892/or. 2012.2184. Epub 2012 Dec 13. PMID: 23242072.
7. Ashrafi M, Sadatmahalleh SJ, Akhoond MR, Talebi M. Evaluation of Risk Factors Associated with Endometriosis in Infertile Women. Int J Fertil Steril. 2016;10(1):11-21. doi: 10.22074/ijfs.2016.4763. Epub 2016 Apr 5. PMID: 27123195; PMCID : PMC4845520.
8. Khan KN, Kitajima M, Fujishita A, et al. Pelvic pain in women with ovarian endometrioma is mostly associated with coexisting peritoneal lesions. Hum Reprod. 2013;28(1):109-18. doi: 10.1093/humrep/des364. Epub 2012 Oct 28. PMID: 23108348.
9. Anaf V, Simon P, Fayt I, Noel J. Smooth muscles are frequent components of endometriotic lesions. Hum Reprod. 2000;15(4):767-71. doi: 10.1093/humrep/15.4.767. PMID: 10739817.
10. Nazri HM, Imran M, Fischer R, et al. Characterization of exosomes in peritoneal fluid of endometriosis patients. Fertil Steril. 2020;113(2):364-373.e2. doi: 10.1016/j.fertnstert.2019.09.032. PMID: 32106990; PMCID: PMC7057257.
11. Chiantera V, Abesadze E, Mechsner S. How to understand the complexity of endometriosis-related pain. Journal of Endometriosis and Pelvic Pain Disorders. 2017;9(1):p.30-8. doi: 10.5301%2Fje.5000271
12. Chapron C, Santulli P, de Ziegler D, et al. Ovarian endometrioma: severe pelvic pain is associated with deeply infiltrating endometriosis. Hum Reprod. 2012;27(3):702-11. doi: 10.1093/humrep/der462. Epub 2012 Jan 16. PMID: 22252082.
13. Samreen N, Bookwalter CA, Burnett TL, et al. MRI of endometriosis: a comprehensive review. Applied Radiology. 2019;5-12.
14. Somigliana E, Vigano P, Benaglia L, et al. Adhesion prevention in endometriosis: a neglected critical challenge. J Minim Invasive Gynecol. 2012;19(4):415-21. doi: 10.1016/j.jmig.2012.03.004. Epub 2012 May 8. PMID: 22575862.
15. Guo SW, Du Y, Liu X. Endometriosis-derived stromal cells secrete thrombin and thromboxane a2, inducing platelet activation. Reprod Sci. 2016;23(8):1044-52. doi: 10.1177/1933719116630428. Epub 2016 Feb 22. PMID: 26902428.
16. Zhang Q, Duan J, Liu X, Guo SW. Platelets drive smooth muscle metaplasia and fibrogenesis in endometriosis through epithelial-mesenchymal transition and fibroblast-to-myofibroblast transdifferentiation. Mol Cell Endocrinol. 2016;428:1-16. doi: 10.1016/j.mce.2016.03.015. Epub 2016 Mar 15. PMID: 26992563.
17. Shi LB, Zhou F, Zhu HY, et al. Transforming growth factor beta1 from endometriomas promotes fibrosis in surrounding ovarian tissues via Smad2/3 signaling. Biol Reprod. 2017;97(6):873-882. doi: 10.1093/biolre/iox140. PMID: 29136085.
18. Kim HS, Yoon G, Ha SY, Song SY. Nodular smooth muscle metaplasia in multiple peritoneal endometriosis. Int J Clin Exp Pathol. 2015;8(3):3370-3. PMID: 26045871; PMCID: PMC4440180.
19. Ibrahim MG, Sillem M, Plendl J, et al. Arrangement of myofibroblastic and smooth muscle-like cells in superficial peritoneal endometriosis and a possible role of transforming growth factor beta 1 (TGFβ1) in myofibroblastic metaplasia. Arch Gynecol Obstet. 2019;299(2):489-99.

doi: 10.1007/s00404-018-4995-y. Epub 2018 Dec 7. PMID: 30523440.

20. Matsuzaki S, Darcha C. Epithelial to mesenchymal transition-like and mesenchymal to epithelial transition-like processes might be involved in the pathogenesis of pelvic endometriosis. Hum Reprod. 2012;27(3):712-21. doi: 10.1093/humrep/der442. Epub 2012 Jan 2. PMID: 22215621.
21. Matsuzaki S, Canis M, Darcha C, et al. Increased mast cell density in peritoneal endometriosis compared with eutopic endometrium with endometriosis. Am J Reprod Immunol. 1998;40(4): 291-4. doi: 10.1111/j.1600-0897.1998.tb00420.x. PMID: 9784802.
22. Matsuzaki S, Canis M, Darcha C, et al. Fibrogenesis in peritoneal endometriosis. A semi-quantitative analysis of type-I collagen. Gynecol Obstet Invest. 1999;47(3):197-9. doi: 10.1159/000010094. PMID: 10087417.
23. Odagiri K, Konno R, Fujiwara H, et al. Smooth muscle metaplasia and innervation in interstitium of endometriotic lesions related to pain. Fertil Steril. 2009;92(5):1525-31. doi: 10.1016/j.fertnstert.2008.08.101. Epub 2008 Oct 18. PMID: 18930216.
24. Guo SW, Ding D, Geng JG, et al. P-selectin as a potential therapeutic target for endometriosis. Fertil Steril. 2015;103(4):990-1000.e8. doi: 10.1016/j.fertnstert.2015.01.001. Epub 2015 Feb 11. PMID: 25681855.
25. Ding D, Liu X, Duan J, Guo SW. Platelets are an unindicted culprit in the development of endometriosis: clinical and experimental evidence. Hum Reprod. 2015;30(4):812-32. doi: 10.1093/humrep/dev025. Epub 2015 Mar 3. PMID: 25740881.
26. Alarcon-Martinez L, Yilmaz-Ozcan S, Yemisci M, Schallek J, Kılıç K, Can A, Di Polo A, Dalkara T. Capillary pericytes express α-smooth muscle actin, which requires prevention of filamentous-actin depolymerization for detection. Elife. 2018;7:e34861. doi: 10.7554/eLife.34861. PMID: 29561727; PMCID: PMC5862523.