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# Correlation between Estrogen Receptorβ (ERβ), Neurofilament Protein (NF), and Protein Gene Product 9.5 (PGP9.5) Expressions as a Marker of Pain on Adenomyosis Etiopathogenesis

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**Abstract**

Adenomyosis is a pathological condition characterized by the presence of endometrial glands and stroma within the myometrium. Endometrial like cells development was influenced by local inflammatory reactions, increases local estradiol due to asynchromatized estrogen synthesis (ERβ) and then stimulated to proliferation and fibrosis, are also irritation on small nerve fibers in women with painful characterized symptoms on adenomyosis. There are expressions of neurofilament protein (NF) and protein gene product 9.5 (PGP 9.5) is a highly specific pan-neuronal marker for microfilament nerve fibers and is related to presenting pain with adenomyosis symptoms. A retrospective immunohistochemical study of thirty samples histopathological of adenomyosis as study groups and 30 with control groups between 25–49 ages which were established at Dr. Hasan Sadikin General Hospital and the satellites in April 2014–May 2015. This case-control analyzed ERβ, NF, and PGP 9.5 expressions compared and correlation between study groups and controls. The results showed there were significant differences in expression of ERβ, NF, and PGP 9.5 on adenomyosis higher than the control study ($p<0.05$). The intensity was higher and very strong into the study groups ($p<0,001$). Cut off point of ERβ was more than>6(sensitivity 66.7%; specificity 70%), NF>3 (sensitivity 93%; specificity 46%), PGP 9.5>4 (sensitivity 90%; specificity 67%). Odds Ratio (CI 95%) ERβ>6= 4.67; NF >3=12.25; PGP 9.5 >4=24.75 ($p<0.001$). The value of histoscore of ERβ and PGP9.5 have correlates to adenomyosis, the differences were statistically significant ($p<0.05$). The conclusion were that the adenomyosis had higher ERβ, NF, and PGP 9.5 expressions. There are simultant correlates and positive values between ERβ, NF, and PGP9.5 based on etiophatogenesis of pain on adenomyosis.

**Keywords**: Adenomyosis, estrogen receptors β(ERβ), neurofilament protein (NF), protein gene product 9.5 (PGP9.5)

# Hubungan antara Ekspresi Reseptor Estrogen β (ER-β), Protein Neurofilamen (NF), Protein Produk Gen 9.5 (PGP 9.5) Sebagai Penanda Nyeri pada Adenomiosis Etiopathogenesis

**Abstrak**

Adenomiosis merupakan keadaan patologis diakibatkan invasi progresif kelenjar dan stroma endometrium ke dalam lapisan miometrium. Keadaan ini dipicu gangguan sintesis estrogen yang mampu meningkatkan estradiol secara berlebih, menyebabkan proliferasi jaringan dan fibrosis, menimbulkan nyeri akibat iritasi serabut saraf tidak bermielin, dapat diamati melalui ekspresi protein neurofilamen dan protein produk gen 9.5. Penelitian analisis laboratorik immunohistokimia komparatif dengan rancangan kasus kontrol pada kelompok kasus dan kontrol masing-masing 30 sampel, rentang usia 25–49 tahun di Rumah Sakit Dr. Hasan Sadikin Bandung dan rumah sakit jejaringnya sejak April 2014 sampai dengan Maret 2015. Penelitian ini menganalisis perbandingan dan hubungan ekspresi ERβ, NF, dan PGP 9.5 antara kedua kelompok. Hasil penelitian menunjukkan ekspresi ERβ, NF dan PGP9.5 pada kelompok kasus lebih tinggi dibanding dengan kontrol ($p<0,05$). Intensitas ekspresi PGP9.5 lebih kuat ($p<0,001$) dibanding dengan kontrol. Berdasar atas histoskor, *cut-off point* ERβ >6 (sensitivitas 66,7%, spesifisitas 70%); NF>3; sensitivitas 93%; spesifisitas 46,7%, PGP9.5 >4; sensitivitas 90%; spesifisitas 67%. OR (IK95%) ERβ>6=4,67; NF>3=12,25; PGP9.5 >4=24,75 ($p<0,001$). Histoskor Erβ dan PGP 9.5 mempunyai korelasi secara simultan dengan adenomiosis ($p<0,05$). Simpulan, terdapat peningkatan ekspresi ERβ, NF, dan PGP 9.5 pada adenomiosis, dan terdapat korelasi simultan serta korelasi positif ERβ dengan NF dan PGP 9.5 sebagai petanda etiopatogenesis nyeri pada adenomiosis.

**Kata kunci**: Adenomiosis, reseptor estrogenβ (ERβ), protein neurofilamen(NF), protein produk gen 9.5 (PGP 9.5)

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## Introduction

Adenomyosis, a progressive gynecologic condition, which is characterized by uterus enlargement and microscopically by the presence of ectopic endometrial gland and stroma surrounded by hyperplastic and hypertrophy myometrium, inciting chronic inflammation and manifest with pelvic pain.[1,2]

The ethiopathogenesis of adenomyosis is still debated and not yet fully elucidated. Among proposed theories, the theory of stromal invasion and endometrial gland into myometrial layer is the most widely accepted theory. The exact prevalence is not known because to make definitive diagnosis of adenomyosis, it requires examination of surgical specimen histopathologically.[1,3]

Adenomyosis is reported in 10% of women in premenopausal age, however it occurs most frequently in reproductive age women (between 25 to 49 of age).[3]

Based on the prevalence of adenomyosis around the world, a figure around 10% is reported, it is assumed that 70 million of women around the world have pelvic pain related to adenomyosis. [3] Hormonal factor appears to play role in the development of adenomyosis, estrogen as tissue proliferation factor. Ectopic endometrial tissue has been shown to express estrogen receptor B (ERB) more prominent than normal tissue and some degree of progeserone resistance also observed.[4]

Adenomyosis generated pain results from chronic irritation of small nerve fibers in myometrial layer. There are correlations between Protein gen product 9.5 (PGP 9.5) and neurofilament protein (NF), which can be detected by using monoclonal antibody in nerve fiber of adenomyosis tissue, and this has correlation with pain manifestation in adenomyosis.[6]

Hyperestrogen state as a result of elevated E2 level which causes excessive proliferation, with expression of both NF protein and PGP 9.5 in non-myelinated nerve fibers found in junctional zone, we suspect a correlation occurs between chronic pelvic pain in the presence of adenomyosis.[6,7] The aim of this study is to compare the relationship between estrogen (ER-β) with the expression of neurofilament protein (NF) and protein gene products 9.5 (PGP 9.5) in nerve fibers of adenomyosis tissue.

## Methods

Subjects in this study is a group of 25–49 aged woman with histopathologically diagnosed adenomyosis as case and with histopathological diagnosis of normal uterus as control. Other histopathologic abnormalities such as endometritis, myoma, and malignancy are excluded.

Sample selection based on histopathological diagnosis after laparotomy surgery. The disease is graded and a sample is taken from the adenomyosis tissue for histopathological examination. Histopathological examination and histochemical analysis were carried out in the Anatomical Pathology Laboratory of Dr. Hasan Sadikin Hospital.

Based on type, it is clinical laboratory research. Based on substance, it is basic and applied research. Based on the relationship among variables studied, it is analytic research. Based on study design, it is categorized as case control study. This is observational analytic with case-control design. Patients with histopathological results of adenomyosis as the case group and patients with histopathological results of non adenomyosis as the control group.

This study utilizes unpaired numeric comparative statistic test between two groups with formula :

$$n = 2\ \frac{\{(Z\alpha + Z\beta)SD^2\}}{X_1 - X_2}$$

Where n is number of sample, SD is standard deviation, α is significance 0.05, Zα=1.65, 1-β is power=0.9, zβ=1.28, and $x_1$-$x_2$ is average difference in histoscore value of estrogen receptor B in adenomyosis and non adenomyosis tissue

Based on a confidence interval of 95% and power of test of 90%, a minimum sample size of 27 was obtained. Our study recruited 30 samples for each case and control. Research was conducted in Department of Obstetric and Gynecology RSUP Dr. Hasan Sadikin Bandung between April 2014 until March 2015.

Statistical analysis used in this study includes normality test use One Sample Kolmogorov Smirnov test. Mann Whitney test used to compare estrogen receptor, neurofilament protein, and protein gene product 9.5 if the data are not normally distributed and independent t-test used if the data are normally distributed. Multiple logistic regression to control confounding variables. This study has obtained

permission from the Health Research Ethics Committee and the Head of the Department of Obstetrics and Gynecology FKUP/RSHS No. 872/UN6.C.10/PN/2017.

## Results

Study examining correlation between estrogen receptor B expression, neurofilament protein and PGP 9.5 as a marker of etiopathogenesis of pain in adenomyosis patient has been done on 30 histopathologic specimen of adenomyosis (as case group) and 30 histopathologic specimen of normal uterus (as control). Immunohistochemistry staining were targeted on estrogen receptor β (ERβ), neurofilament protein (NF), and protein gen product 9.5 (PGP 9.5).

Subject characteristic in this study is the age of participants. There is significant difference in terms of age between two studied groups ($p<0.05$), with relatively young age in the case group (median 45) compared to control (median 47.5). 83% of subjects in control groups are over 45 years old. Because there is a significant difference, age is considered as confounding variables and requires further analysis.

Expression of estrogen receptor β (ERβ), neurofilament protein (NF), and protein gen product (PGP 9,5) in both groups are analyzed immunohistochemically based on intensity and distributions as shown in Table 1. There is a difference in intensity and distribution of Erβ between group studies. For the case group, the median is strong, while the moderate category is observed in the control group. In terms of distribution, there is no significant difference observed ($p>0.05$).

Studies on intensity and distribution of NF between two groups revealed significant differences ($p<0.05$). In terms of intensity in the case group, median is categorized as moderate, while weak in the control group. For distribution of NF, most cases group are > 50–80% (47%), while control group most distribution falls on 20–50% (57%) .

PGP 9.5 results revealed significant differences with regard to its intensity and difference between two comparison groups ($p<0.001$). PGP 9.5 mostly expresses strong to very strong intensity in the case group, however negative and weak expressions are observed in the control group. Distribution of PGP 9.5 in the case group mostly falls between >50–80% (73%), while in the control group range falls between 20–50% (57%).

From the intensity and distribution result, histoscore can be calculated by multiplying

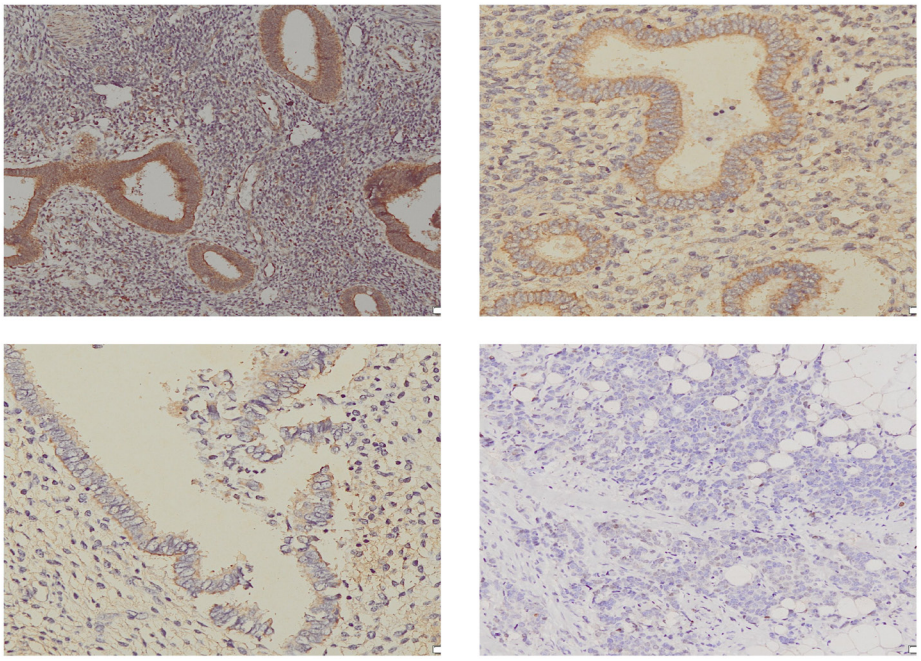

**Figure Low to Very Strong Intensity on PGP 9.5, ERB and NF with Immunohistochemical Staining**

**Table 1 Expression of Estrogen Receptor β (ERβ), Neurofilament Protein (NF), and Protein Gen Product 9.5 (PGP 9.5) in Adenomyosis and Control Group**

| | Group | | P-Value* |
|---|---|---|---|
| | Case (n=30) | Control (n=30) | |
| Reseptor estrogen B | | | |
| Intensity | | | <0.001 |
| Negative | 0 | 4 | |
| Weak | 4 | 11 | |
| Moderate | 4 | 11 | |
| Strong | 12 | 2 | |
| Very strong | 10 | 2 | |
| Distribution | | | |
| Median | 50 | 40 | |
| Range | 15–85 | 15–85 | 0.194 |
| ≤20% | 3 | 6 | |
| 20–50% | 12 | 14 | |
| >50–80% | 12 | 7 | |
| >80% | 2 | 3 | |
| Neurofilamen protein (NF) | | | |
| Intensity | | | <0.001 |
| Negative | 0 | 12 | |
| Weak | 9 | 11 | |
| Moderate | 12 | 5 | |
| Strong | 7 | 2 | |
| Very strong | 2 | 0 | |
| Distribution | | | 0.047 |
| Median | 57.5 | 37.5 | |
| Range | 15–85 | 15–85 | |
| ≤2% | 3 | 5 | |
| 20–50% | 10 | 17 | |
| >50–80% | 14 | 7 | |
| >80% | 3 | 1 | |
| Protein PGP 9.5 | | | |
| Intensity | | | <0.001 |
| Negative | 0 | 15 | |
| Weak | 6 | 8 | |
| Moderate | 8 | 3 | |
| Strong | 6 | 3 | |
| Very strong | 10 | 1 | |
| Distribution | | | <0.001 |
| Median | 65 | 30 | |
| Range | 20–85 | 15–85 | |
| ≤ 20% | 1 | 8 | |
| 20–50% | 5 | 17 | |
| >50–80% | 22 | 4 | |
| >80% | 2 | 1 | |

Note: *) based on Mann-Whitney test

intensity (I+1) with distribution. Results are shown in Table 2. Histoscore of ERB, NF, and PGP 9.5 calculated based on median are different between two groups in comparison. In the case group, the histoscore of the three variables are higher compared to control. Based on the difference in the result of the histoscore of ERB, NF, and PGP 9.5, we can determine cut off value which can be used to correlate pain etiopathogenesis in adenomyosis. Statistical

**Table 2 Expression of ERβ, NF and PGP9.5 based on Histoscore in Case and Control Group**

| Histoskor | Group | | P-Value* |
|---|---|---|---|
| | Case (n=30) | Control (n=30) | |
| ERB | | | 0.001 |
| Median | 8.5 | 6 | |
| Range | 4–16 | 2–16 | |
| NF | | | <0.001 |
| Median | 8 | 4 | |
| Range | 3–16 | 1–16 | |
| PGP9.5 | | | <0.001 |
| Median | 9.5 | 2 | |
| Range | 4–15 | 1–16 | |

analysis with the ROC curve showed a cut-off point for ERB > 6 with sensitivity of 66.7% and specificity of 70%. For NF cut-off point >3 has 93% sensitivity and specificity of 46.7%. With regard to PGP 9.5, cut-off >4 has sensitivity of 90% and 67% specificity.

Based on a cut off point determined in table 3, Odds Ratio (OR) can be calculated with a confidence interval of 95%. From the Table 3, OR for ERB >6 is 4,67; OR for NF >3 is 12,25 and for PGP 9.5 >4 is 24.75. Odds ratio for ERB is statistically significant, while for NF and PGP 9.5 is very significant (p<0.001).

We further analyze the data using binary logistic regression model, and the correlation between age as confounding variable, with ERB, NF, and PGP 9.5 histoscore as marker of pain etiopathogenesis in the incidence of

**Table 3 Association between Cut-off Point of ERB, NF dan PGP 9.5 Histoscore and the Incidence of Adenomyosis**

| Cut-off Point | Group | | P-Value* | OR (IK 95%) |
|---|---|---|---|---|
| | Case (n=30) | Control (n=30) | | |
| ERB | | | 0.004 | 4.67 (1.57–13.87) |
| >6 | 20 | 9 | | |
| ≤6 | 10 | 21 | | |
| NF | | | <0.001 | 12.25 (2.46–60.91) |
| >3 | 28 | 16 | | |
| ≤3 | 2 | 14 | | |
| PGP 9.5 | | | <0.001 | 24.75 (2.1 –18.87) |
| >4 | 27 | 8 | | |
| ≤ 4 | 3 | 22 | | |

Note: OR(IK95%)

**Table 4 Multivariate Analysis Result in Determining Correlation between Estrogen Receptor B, Neurofilament Protein, and Protein Gen Product 9.5 as Etiopathogenesis Marker of Pain in Adenomyosis by Using Binary Logistic Regression Model**

| Variables | Coefficient B | Error Standart B | P-Value | OR (IK 95 %) |
|---|---|---|---|---|
| Early model: | | | | |
| Age (<45) | 1.439 | 0.869 | 0.098 | 4.22 (0.77–23.14) |
| Histoscore ERB (> 6) | 0.582 | 0.857 | 0.497 | 1.79 (0.33–9.61) |
| Histoscore NF (>3) | 2.197 | 1.015 | 0.030 | 9.00 (1.23–65.76) |
| Histoscore PGP9.5>4 | 2.402 | 0.899 | 0.008 | 11.04 (1.89–64.32) |
| Final model: | | | | |
| Histoscore NF (>3) | 1.936 | 0.942 | 0.040 | 6.93 (1.09–43.92) |
| Histoscore PGP9.5>4 | 2.909 | 0.765 | <0.001 | 18.33 (4.09–82.12) |

Note: model accuration=85%; $R^2$=0,551

**Table 5 Correlation Between Variabels Measured in Adenomyosis Case and Control**

| Correlation | Case | | Control | |
|---|---|---|---|---|
| | Correlation coefficient ($r_s$) | P-Value | Correlation coefficient ($r_s$) | P-Value |
| Age and histoscore ERB | 0.153 | 0.419 | -0.032 | 0.867 |
| Age and histoscore NF | 0.134 | 0.481 | 0.389 | 0.033 |
| Age and histoscore PGP 9.5 | -0.073 | 0.703 | 0.012 | 0.012 |
| Histoscore ERB and histoscore NF | 0.026 | 0.890 | 0.165 | 0.384 |
| Histoscore ERB and histoscore PGP 9.5 | 0.475 | 0.008 | 0.476 | 0.008 |
| Histoscore NF and histoscore PGP 9.5 | 0.029 | 0.897 | 0.074 | 0.698 |

Note: $r_s$=rank spearman correlation coefficient

adenomyosis as shown in Table 4.

From Table 4, based on four variable tested in analytical there are two variables have significant correlation with adenomyosis, that is histoscore NF (>3) with OR (IK 95%); 6.93 (1.09–43.92) and for histoscore PGP9.5 (>4) OR (IK 95%); 18.33 (4.09–82.12). Age <45 years has a risk of 4,22 times associated with the expression of the three variables.

To find the degree of correlation from different variables measured in case and control group, as shown by Table 5. There is significant correlation between ERB histoscore and PGP 9.5 histoscore with moderate Guilford correlation of r=0.475 observed in the case group. Meanwhile in the control group, beside correlation between ERB histoscore and PGP 9.6 histoscore, we also observed correlation between age and NF, with correlation of 0.476 and 0.389 respectively.

Based on the result presented in Table 5, there is significant correlation between ERB histoscore and PGP 9.5 histoscore with moderate Guilford correlation of r=0.475 observed in the case group. Meanwhile in the control group, beside correlation between ERB histoscore and PGP 9.6 histoscore, we also observed correlation between age and NF, with correlation of 0.476 and 0.389 respectively.

## Discussion

Theory explaining exact etiology of adenomyosis until now remains mystery and not yet elucidated. However the stromal and endometrial gland invasion into myometrial layer is widely accepted nowadays.[8,9] Studies showed estrogen role in the etiopathogenesis of adenomyosis, such as fibrosis of adenomyosis tissue due to excess of local estrogen level, because of the absent of 17BHSD2 enzyme so estrogen could not be converted back to estrone, progesterone resistance, further leading to local inflammation through activation of pro-inflammatory cytokine.[4,8,12,13] There are variations in the clinical feature of the disease, from asymptomatic until severe symptoms like dysmenorrhea, dyschezia, dyspareunia, chronic pelvic pain, and infertility. Pain results from local inflammation and irritation of unmyelinated small nerve fibers in myometrial junctional zone and endometrium below.[5,14,17,18] Zhang, Li studied the correlation between neurofilament protein and PGP 9.5 expression of nerve fiber in myometrium with adenomyoma and considered the correlation with pain etiopathogenesis in adenomyosis. Our study showed higher expression of estrogen receptor β, neurofilamen protein, and PGP 9.5 in the case group compared to control. Other researchers also affirm our result. Aromatase has been shown to convert androstenedion to estradiol, so adenomyosis tissue has the ability to synthesize local estrogen which can lead to progressivity of adenomyosis lesion.[4,8,10,11,13]

Meanwhile in the control group, expression of estrogen receptor β is so low hence the endometrium is unable to synthesize local estradiol. Endometrial proliferation in this condition is driven by peripheral estradiol which is produced by the ovary, synergystically following ovulatory cycle. Several theories showed there is balance expression between estrogen receptor β and alpha, however in this study there is preponderance toward strong estrogen receptor β expression. This proved that in adenomyosis there are alterations in estrogen metabolism, disturbance in aromatase, 17BHSD2 deficiency, progesteron resistance, leading to uncontrolled proliferation of adenomyosis lesion in myometrial layer. [4,8,10,11,13]

Pain mechanism in adenomyosis is stimulated by local injury which irritate unmyelinated nerve

fibers for prolonged duration, resulting in chronic pelvic pain and dysmenorrhea, as complained by most women. Nerve fiber in adenomyosis tissue express neurofilamen protein and protein gen product 9.5 specifically and could not be found in normal tissue.[5,17,18] Their expression can also be found weakly in some cases of intramuscular myoma.[17,19] In this study, as reported by Zhang and Tokushige, expression of both proteins are found in strong intensity and proved their role in etiopathogenesis of pain in adenomyosis. That found low to moderate expression of both protein in the control group. It is suspected that there are micromatous lesion in control tissue.

Based on multivariate analysis in the search of pain ethiopathogenesis with the risk of developing adenomyosis, PGP 9.5 has the most significant correlation. This agrees with previous studies which indicate expression of PGP 9.5 as a specific marker for pain pathogenesis in adenomyosis. Also we observed strong correlation between ERβ expression with PGP 9.5 but not significant statistically. Pain ehiopathogenesis in adenomyosis could be meaningful if both expressions of ERβ and PGP 9.5 are present in adenomyosis. Further studies are needed to find correlation between pain intensity as reported clinically.

In this study, age less than 45 has odds of 4.22 for the expression of three variables of pain ethiopathogenesis marker in adenomyosis, and has strong correlation with neurofilament protein expression. Pain in adenomyosis can manifest as dysmennorhea, dysuria, dyschezia, and dyspareunia, mostly complained by women over 35 years of age, with regard to infertility problem, it affects women in active reproductive age.[2,20,21]

Limitation of this study is the small number of subjects enrolled, related to strict inclusion criteria, difficulty in acquiring adenomyosis tissue because of its low incidence, and difficult technique in immunohistochemistry staining. Another aspect is the use of secondary data so there is no information on the degree of pain, nor the history of hormonal preparation use which can confound independent variables being tested.

Expression of Estrogen receptor β (ERβ), protein neurofilamen protein (NF), and protein gen product 9.5 (PGP 9.5) in adenomyosis tissue is significantly higher in the case group compared to control. Expression of neurofilamen protein (NF) and protein gen product 9.5 (PGP 9.5) have stimultaneous correlation with Estrogen receptor β (ERβ) in the incidence of adenomyosis. From bivariate and multivariate analysis, expression of PGP 9.5 has the strongest correlation with etiopahogenesis of pain in adenomyosis. There is positive correlation between ERβ histoscore and PGP 9.5 histoscore in case group ($r=0.475$)/($p<0.008$), and strong correlation between etiopathogenesis of pain in adenomyosis.

Further studies are needed using pain intensity scale in adenomyosis patient. Research in role of Prostaglandin, COX, 17BHSD2 and their correlation with NF and PGP 9.5 as etiopathogenis marker in pain are needed. Strong correlation between ERβ and PGP 9.5 and etiopathogenesis of pain in adenomyosis can explain the nature of chronic pelvic pain in adenomyosis, related to progesteron therapy and provide information in the development of guideline in managing adenomyosis related pain.

## References

1. Speroff L, Fritz MA. Adenomyosis, editors. In: Clinical Gynaecologi Endocrinology and Infertility. 8th ed. Philadelphia: Lippincott Williams and Walkins; 2011.
2. Mc Williams MM, Chennatukuzhi VM. Recent advances in uterine fibroid etiology. Semin Reprod J. 2017;35(2):181–9.
3. Taran FA, Stewart EA, Brucker S. Adenomyosis: Epidemiology risk factors clinical phenotypes and surgical interventional alternatives to hysterectomy. Geburtshiife und Frauenhelkunde. 2013;73(9):924–31.
4. Benagiano G, Habiba M, Brosen I. The pathophysiology of uterine adenomyosis : an update. Fertil Steril. 2012;98(3):572–9.
5. Novella M, Harraiz S. The effect of antiangiogenic treatment on peritoneal endometriosis-Associated nerve fibers. Fertil Steril. 2012;98:1209–17.
6. Wang Wen-Li, Hua D, Zhang. Expression of PGP95, NF and their reationship with dysmenorrhea in patients with adenomyosis. J Capital Medical Univ. 2012;(3):12.
7. Barcena de Arellano ML, Oldeweme J, Arnold J, Schneider A, Mechsner S. Remodeling of estrogen-dependent sympathetic nerve fibers seems to be disturbed in adenomyosis. Fertil Steril. 2013;100(3):801–9.
8. Jean Baptise H, Tetrokalashvili M, Williams T, Fogel J. Characteristic associated with postoperative diagnosis of adenomyosis or combined adenomyosis with fibroids. Int J

Gynecol Obstet. 2013;122(2):112–4.
9. Vannucini S, Petraglia F. Recent advances in understanding and managing adenomyosis. Fertil Steril. 2019;8:283.
10. Mehasseb MK, Panchal R, Taylor AH, Brown L, Bell SC, Habiba M. Estrogen and progesterone receptor isoform distribution through the menstrual cycle with and without adenomyosis. Fertil Steril. 2011;95(7):2228–35.
11. Badawy AM, Elnasher AM, Mosbah AA. Aromatase inhibitors or gonadotropinj releasimh hormone agonists for management of uterine adenomyosis: randomized controlled trial. Acta Obstet Gynecol Scand. 2012;91:489–95.
12. Khreisat B, Al-Rawabeh, Duquom W, Al-Qudah M. Adenomyosis: frequency of hysterectomy in histopathological specimens at two Jordanian Mil Hospitals. JRMS; 2011; 18 (2):76–9.
13. Al-Sabbagh M, Lam EW, Brosens JJ. Mechanism of endometrial progresterone resistance. J Mol Cell Endocrinol. 2012;358:208–15.
14. Pistofidis G, Makrakis E, Koukoura O, Bardis N, Balinakos P, Anaf V. Distinct types of uterine adenomyosis based on laparoscopic and histopatologic criteria. Clin Exp Obstet Gynecol. 2014;41(2):113–8.
15. Plante BJ, Lessey BA, Taylor RN, Wang W, Bagchi MK, Yuan L, et al. G protein coupled estrogen receptor (GPER) expression in normal and abnormal endometrium. Reprod Sci. 2012;19(7):684–93.
16. Tian R, Wang Z, Shi Z, Li D, Wang Y, Zhu Y, et al. Differential expression of G-protein coupled estrogen receptor 30 in human uterine myometrial smooth muscle. Fertil Steril. 2013;99(1):256–63.
17. Choi YJ, Chang JA, Kim YA, Chang SH, Chun KC, Koh JW. Innervation in women with uterine myoma and adenomyosis. Obstet Gynecol Sci. 2015;58(2):150–6.
18. Li Y, Zhang S, Xu L. Expression of nerve growth factor produced by ectopic endometrium from patients with adenomyosis and its relationship with pain scales and innervation. J Fertil Sterile. 2014;27:287–91.
19. Hendry D, Madjid HT, Ruswana A, Rachmawati A. Correlation expression immunocytochemistry vascular endothelial growth factor A (VEGFA) with protein gene product 9.5 (PGP 9.5) of menstrual blood on pathophysiologi endometriosis. Andalas Obstet Gynecol J. 2017;1(1):38–46.
20. Vinci V, Saldari M, Sergie E, Bernardo S, Rizzo G, Porpora G et al. Imaging MRI US or real time virtual sonography in the evaluation of adenomyosis. Radio Med J. 2017;122(5):361–8.