# The Correlation Between CYP19 Levels, VAS of Dysmenorrhea, and the Grading Score ASRM of Endometriosis

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**Abstract**
**Objective**: We aim to assess the correlation between the CYP19 level, the visual analogue scale (VAS) pain and endometriosis American Society for Reproductive Medicine (ASRM) grading scale.
**Method**: An observational analytic study with a cross–sectional design was conducted at the Obstetrics/ Gynecology Department, RSUP Dr. Moh Hoesin Palembang, Laboratory of Biomedical and Biomolecular of the Faculty of Medicine, Universitas Sriwijaya from July–December 2022. Subjects who were diagnosed with endometriosis and met the inclusion and exclusion criteria underwent a histopathological examination to measure the degree of disease according to ASRM. Using the same sample, the CYP19 levels were assessed by enzyme linked immunosorbent assay (ELISA). Univariate and bivariate statistical analyses were performed to see the correlation.
**Results**: This study involved 37 subjects aged 20–48 (Mean: 32 years). We found there was a significant correlation between the degree of VAS score and ASRM grading ($p<0.001$). In addition, although CYP19 expression was found in ectopic endometrial tissue, there was no significant correlation between the level of CYP19 expression in the tissue and the VAS score dysmenorrhea ($p=0,618$) and ASRM grading ($p=0.604$).
**Conclusion**: There is no significant correlation between CYP19 levels and the degree of pain in VAS or ASRM grading in patients.

**Key word**: ASRM, CYP19 Levels, Endometriosis

# Hubungan Kadar *CYP19, Visual Analogue Score* (VAS) Dismenorea, dan *Grading Score American Society for Reproductive Medicine* (ASRM) Endometriosis

**Abstrak**
**Tujuan**: Penelitian ini bertujuan untuk menilai hubungan kadar CYP19, skala nyeri visual analogue scale (VAS) dan grading berdasarkan American Society for Reproductive Medicine (ASRM) pada pasien endometriosis.
**Metode**: Sebuah studi analitik observasional dengan rancangan cross–sectional dilakukan di Bagian/KSM Obstetri Ginekologi, RSUP Dr. Moh Hoesin Palembang dan Laboratorium Biomedis dan Biomolekuler Fakultas Kedokteran Universitas Sriwijaya, pada periode Juli–Desember 2022. Subjek penelitian yang terdiagnosis endometriosis dan memenuhi kriteria inklusi dan eksklusi, dilanjutkan pemeriksaan histopatologis untuk mengukur derajat penyakit sesuai rASRM. Dengan menggunakan sampel yang sama dilakukan penilaian kadar CYP19 melalui pemeriksaan enzyme linked immunosorbent assay (ELISA). Analisis statistik univariat dan bivariat dilakukan untuk melihat hubungan tersebut.
**Hasil**: Penelitian ini melibatkan 37 subjek dengan rentang usia 20–48 (Median: 32 tahun). Pada penelitian, terdapat hubungan yang bermakna antara derajat nyeri VAS endometriosis terhadap derajat endometriosis ($p<0,001$). Selain itu, walaupun ditemukan adanya ekspresi CYP19 pada jaringan endometrium ektopik, namun tidak terdapat hubungan yang bermakna antara tingkat kadar CYP19 pada jaringan dengan derajat VAS score dismenorea ($p=0,618$) dan derajat ASRM endometriosis ($p=0,604$).
**Kesimpulan**: Tidak terdapat hubungan bermakna antara kadar CYP19 dengan derajat nyeri VAS maupun derajat ASRM endometriosis pada pasien.

**Kata kunci**: ASRM, Kadar CYP19, Endometriosis

## Introduction

Endometriosis is a gynecological disorder due to the growth of ectopic endometrial tissue, the estimated prevalence of about 10–15% in women of reproductive age (age 15–44 years), with 70% of them experiencing severe pelvic pain–generally measuring the degree of pain using a visual analog scale (VAS) – requires surgical evaluation and histopathological examination.[1–3] Based on its examination, the classification of endometriosis can be carried out using the American Society of Reproductive Medicine (ASRM) scoring system according to type, location, appearance, depth of invasion of the lesion, the spread of disease and continuous implantation with the clinical symptoms experienced by the patient.[4,5]

The etiology of this disease is still uncertain, causing recent research to start targeting the influence of changes in gene expression involved in the mechanism of endometriosis. Recent literature reveals changes in expression by the CYP19 gene which is found to have high expression in eutopic endometrial tissue of symptomatic endometriosis patients and is probably related to the severity of clinical symptoms of endometriosis.[6–9]

CYP19 (P450arom, aromatase) can be found in endometriosis tissue to catalyze androgen aromatization to estrogen. CYP19 enzyme inhibitors have been used extensively as targets for inhibitor therapy in estrogen–sensitive diseases, including cancer, endometriosis, and leiomyomas.[10] CYP19 enzymes are enzymes involved in the conversion of androgens (such as testosterone) to estrogens (such as 17β–estradiol). CYP19 is a key enzyme as a catalyst in the conversion of adrenal androgens through sequential hydroxylation reactions to become aromatic estrogens.[11,12]

Currently, data related to the relationship between CYP19 expression, and the clinical and histopathological severity of endometriosis are still very limited. Therefore, this study aims to determine the effect of the CYP19 levels with the VAS pain scale and grading based on ASRM in endometriosis patients at RSUP Dr. Moh. Hoesin Palembang.

## Method

An observational analytic study with a cross–sectional design was conducted at the Obstetrics/Gynecology Department, RSUP Dr. Moh Hoesin Palembang, and Laboratory of Biomedical and Biomolecular of the Faculty of Medicine, Sriwijaya University, from July–December 2022. Subjects diagnosed with endometriosis and meeting the inclusion criteria included endometriosis patients who agreed to be involved in the study and the diagnosis was proven by histopathological examination to measure the degree of disease according to ASRM. Sample can be taken from a laparoscope or laparotomy. Meanwhile, endometriosis patients who were undergoing hormone therapy were excluded from this study. Using the same sample, the CYP19 levels were assessed by enzyme linked immunosorbent assay (ELISA). Univariate and bivariate statistical analyses were performed to see the correlation.

## Results

There were 37 subjects aged 20–48 years old (median: 32 years). Based on demographic data, we found there were no significant differences between the mild–grade endometriosis group (35.47±9.002 years) and moderate–severe (34.11±6.902) (p=0.608). Most research subjects graduated from high school (62.2%) and were domiciled outside Palembang (67.6%) (Table 1).

**Table 1 Demographic Data**

| Variable | (n) | (%) |
|---|---|---|
| **Age** | | |
| 20–30 | 15 | 40.5 |
| 31–40 | 13 | 35.1 |
| > 40 | 9 | 24.3 |
| **Education** | | |
| Elementary | 1 | 2.7 |
| High School | 23 | 62.2 |
| Diploma | 5 | 13.5 |
| Bachelor | 8 | 21.6 |
| **Domicile** | | |
| Palembang | 12 | 32.4 |
| Outside Palembang | 25 | 67.6 |

We found median age of the subjects in mild endometriosis group was 35.47±9.002 years and in moderate-severe endometriosis group was 34.11±6.902 years. The median of VAS of mild endometriosis was 3 (1–5) and in moderate-severe endometriosis was 7 (5–8). Measurement of CYP19 expression levels in the mildgrade endometriosis group (0.093 ng/ml; range 0.069–0.402 ng/ml) and moderate–severe endometriosis (0.0905 ng/ml; range 0.007–0.194 ng/ml) found no significant difference (p=0.098) (Table 2).

There were 19 subjects with both mild endometriosis degree and mild VAS score, while 2 subjects have moderate–severe endometriosis degree and mild VAS score. There were no subjects with high VAS in mild endometriosis, but there were 16 subjects with high VAS in moderate–severe endometriosis. In addition, there was a significant correlation between the degree of endometriosis pain to the degree of ASRM endometriosis (p<0.001) (Table 3).

We found 16 subject who have low CYP19 levels and mild endometriosis. There were 17 subjects with low CYP19 levels but had moderate–severe endometriosis. In the mild endometriosis group, there were 3 subjects with high CYP19 levels, while in the moderate–severe endometriosis group, there was 1 subject with high CYP19 levels. Furthermore, although we found the presence of the *CYP19* enzyme in ectopic endometrial tissue, there was no significant correlation between the level of CYP19 expression in the tissue and the degree of endometriosis (p=0.604) (Table 4).

There were 18 subjects with low CYP19 levels and low VAS and there were 3 subjects with low VAS with high CYP19 levels. There were 15 subjects with high VAS at low CYP19 levels, while there was 1 subject with high VAS in the group with high CYP19. However, there was no significant correlation between the level of CYP19 expression in the tissue and the VAS score of dysmenorrhea (p=0.618) (Table 5).

From the Kruskall–Wallis test presented in the table 6, it was found that the degree of endometriosis had significant relationship with the vas score (p<0.001). There was no significant relationship between CYP 19 levels and the VAS score of dysmenorrhea (p=0.442).

**Table 2 Characteristics of Subject**

| Variable | Endometriosis Degree (ASRM Classification) | | | |
|---|---|---|---|---|
| | Mild (n = 19) | | Moderate–Severe (n = 18) | |
| | Mean (SD) | Median (Min–max) | Mean (SD) | Median (Min–max) |
| **Age** | 35.47 (± 9,002) | – | 34.11 (± 6,902) | – |
| **VAS Score** | – | 3 (1–5) | – | 7 (5–8) |
| ***CYP19* (ng/mL) Levels** | – | 0.093 (0.069–0.402) | – | 0.0905 (0.007–0.194) |

**Table 3 The Correlation between VAS Score and ASRM Classification**

| VAS Score | Endometriosis Degree (ASRM Classification) | | Total | p | OR (95%CI) |
|---|---|---|---|---|---|
| | Mild | Moderate–Severe | | | |
| **Mild** | 19 | 2 | 21 | | |
| **Severe** | 0 | 16 | 16 | <0.001 | 257.40 (11.52–5750.39) |
| **Total** | 19 | 18 | 37 | | |

*Chi–square*

**Table 4 The Correlation between CYP19 Levels and The Endometriosis Degree**

| *CYP19 Levels* | Endometriosis Degree (ASRM Classification) | | Total | p | OR (95%CI) |
|---|---|---|---|---|---|
| | Mild | Moderate–Severe | | | |
| **Low** | 16 | 17 | 33 | | |
| **High** | 3 | 1 | 4 | 0.604 | 0.314 (0.030–3.336) |
| **Total** | 19 | 18 | 37 | | |

*Chi–square*

**Table 5 The Correlation between CYP19 Levels and VAS Score of Dysmenorrhea**

| *CYP19 Levels* | VAS Score | | Total | p | OR (95%CI) |
|---|---|---|---|---|---|
| | Mild | Severe | | | |
| **Low** | 18 | 15 | **33** | | |
| **High** | 3 | 1 | **4** | 0.618 | **0.400** (0.038–4.256) |
| **Total** | **21** | **16** | **37** | | |

*Chi–square*

**Table 6 Analysis of the Correlation Between CYP19 Levels, Degree of Endometriosis and the VAS Score**

| Variable | VAS Score | | p |
|---|---|---|---|
| | Mild | Severe | |
| **Endometriosis Degree** | 21 | 16 | <0.001 |
| **CYP19 Levels** | 21 | 16 | 0.442 |

## Discussion

In this study, we involved 37 subjects aged 20–48 years old with a median age of 32 years. There was no significant difference in the age of the research subject in the mild degree of endometriosis group and a moderate–severe endometriosis group (35.47±9.002 years compared to 34.11± 6,902 years; p=0.608). It can be assumed that the age of the subject in the mild degree of endometriosis group and the moderate–severe endometriosis group in this study has similar characteristics. Therefore, in this study, the age of the subject will not be analyzed further.

We found there was a significant relationship between the VAS score and the degree of endometriosis (p<0.001). It corresponds with a study in deep infiltrating endometriosis (DIE) patients which found that there was a positive correlation between the degree of pain and the diameter of the

lesion and the location of the endometriosis. In addition, central nervous sensitization can lead to pain memory formation and hypersensitivity to persistent pain due to peritoneal inflammation in endometriosis. Furthermore, it can be concluded that the severity of endometriosis through ASRM classification and the location of the lesion is related to the degree of pain associated with increased inflammation and sensitization due to lesions and retrograde menstruation.[13,14]

Moreover, we found the presence of CYP19 expression in ectopic endometrial tissue in endometriosis, although there was no significant relationship between the level of CYP19 levels in tissues and the degree of endometriosis (p=0.604). In a study, comparisons were made between endometriosis patients and healthy patients, CYP19 expression was found in endometriosis tissue and this expression was not found in endometrium, myometrium and peritoneal tissues in healthy patients and CYP19 levels did not differ significantly between mild and severe endometriosis.[15]

CYP19 activity is known to increase local biosynthesis of estrogen, which will stimulate prostaglandin E2 (PGE2) production by upregulating cyclooxygenase 2 (COX–2) which forms a positive feedback mechanism that is one of the characteristics of endometriosis. Prostaglandin and estrogen concentrations are associated with proliferation, migration, angiogenesis and apoptosis resistance. Thus, overexpression of CYP19 in endometriosis stromal cells can lead to endometriotic implantation.[8,11,16,17]

In this study, a non–significant relationship of the level of CYP19 levels in tissues and the degree of endometriosis could be caused by several factors. This study used the classification of the degree of endometriosis following the ASRM classification. The degree of endometriosis is based on ASRM considering the extent and depth of the lesion without considering the results of histopathological examination.[18,19] In addition, this study utilized the examination of CYP19 levels originating from tissues using ELISA. Therefore, it was found that CYP19 levels per gram of endometriosis tissue were not significantly different in mild and moderate–severe endometriosis. This study shows that CYP19 levels do not bring useful results to determine the stage of endometriosis. However, further research is needed to ascertain whether CYP19 levels in endometriosis tissue have a clinical correlation with the degree of endometriosis.

We found that there was no significant relationship between the level of CYP19 levels in tissues and VAS score (p = 0,618). However, previous studies revealed that increasing levels of PGE2 and COX–2, which inflammatory cytokine that have role in endometriosis, is might be influenced by the action of the CYP19 enzyme that found in endometriosis tissue.[9,10,20]

Pain in endometriosis is related to the inflammatory process. The inflammation during menstruation that occurs in endometriosis, coupled with the presence of retrograde menstrual residues in the peritoneal cavity, leads to increased levels of PGE2, reactive oxygen species (ROS), and other proinflammatory cytokines such as tumor necrosis factor alpha (TNF–alpha). The relationship between the inflammatory process and CYP19 expression is mediated by NF–kB which is a transcription factor responsible for activating genes associated with inflammatory processes. Increased inflammation in endometriosis shows an increase in estrogen caused by an increase in prostaglandins through activation of COX–2 and nuclear factor–kappa B (NF–kB). [14,21]

## Conclusion

We found there is no significant correlation between CYP19 levels and the degree of pain in VAS score and the degree of endometriosis

in ASRM grading.

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