ORIGINAL ARTICLE

Bali Medical Journal (*Bali MedJ*) 2023, Volume 12, Number 3: 2975-2980
P-ISSN.2089-1180, E-ISSN: 2302-2914

# The correlation between Anogenital Distance (AGD) and Endometriosis

Ricky Ricardo Nalley[1*], Nusratuddin Abdullah[2], Fatmawati Madya[2], St. Nur Asni[3], Sriwijaya[2], Susiawaty[4]

## ABSTRACT

**Background:** Endometriosis is a disease that causes recurrent chronic pain related to menstruation in around 10% of women of reproductive age. However, current non-invasive diagnostic tools are not sufficiently good at detecting endometriosis. Anogenital Distance (AGD) is a new measurement parameter that is non-invasive and proven to be used to help detect endometriosis. This study aims to assess the ability of AGD in detecting endometriosis.

**Methods:** This study is a diagnostic test study involving 35 endometriosis patients and 35 controls by comparing the clinical characteristics of the patients and the length of AGD-AF and AGD-AC for both groups. Data were analyzed using SPSS version 22.0 for Windows.

**Results:** The median AGD-AF length in endometriosis patients and controls were 22.65 $\pm$ 6.54 and 29.53 $\pm$ 3.52 ($p \leq 0.001$) respectively, with AGD-AC lengths being 68.04 $\pm$ 5.8 and 66.81 $\pm$ 5.49 ($p=0.369$). In the multivariate logistic regression test, AGD-AF length was a single significant predictor ($p < 0.001$). Based on the ROC curve, the length of AGD-AF with a cut-off of 23.35 mm has a sensitivity and specificity value of 97.1% and 65.7%.

**Conclusion:** This study states that there is a correlation between AGD and endometriosis, especially AGD-AF, so it can be used as one of the predictors to help detect endometriosis.

**Keywords:** Anogenital Distance, AGD-AF, AGD-AC, Endometriosis.
**Cite This Article:** Nalley, R.R., Abdullah, N., Madya, F., Asni, S.N., Sriwijaya., Susiawaty. 2023. The correlation between Anogenital Distance (AGD) and Endometriosis. *Bali Medical Journal* 12(3): 2975-2980. DOI: 10.15562/bmj.v12i3.4707

[1]Department of Obstetrics and Gynecology, Faculty of Medicine, Universitas Hasanuddin, Makassar, Indonesia;
[2]Fertility and Reproductive Endocrinology Subdivision, Department of Obstetrics and Gynecology, Faculty of Medicine, Universitas Hasanuddin, Makassar, Indonesia;
[3]Urogynecology and Reconstructive Pelvic Surgery Subdivision, Department of Obstetrics and Gynecology, Faculty of Medicine, Universitas Hasanuddin, Makassar, Indonesia;
[4]Social Obstetrics and Gynecology Subdivision, Department of Obstetric and Gynecology, Faculty of Medicine, Universitas Hasanuddin, Makassar, Indonesia.

*Corresponding author:
Ricky Ricardo Nalley;
Department of Obstetrics and Gynecology, Faculty of Medicine, Universitas Hasanuddin, Makassar, Indonesia;
rickyricardonalley@gmail.com

Received: 2023-07-13
Accepted: 2023-09-03
Published: 2023-10-07

## INTRODUCTION

Endometriosis is the presence of endometrial tissue in an abnormal or ectopic location. Histologically, endometriosis is the presence of endometrial-like tissue or glands outside the uterine cavity. Endometriosis is the most frequently observed gynecological disorder in reproductively active women. Ectopic endometrial tissue is responsive to hormonal stimulation. Endometriosis is often associated with dyspareunia, menstrual pain, and pelvic pain. Endometriosis symptoms can negatively impact a patient's quality of life.[1,2]

Worldwide, the estimated prevalence of endometriosis in premenopausal women is as much as 10% and about 30-50% with symptoms. Endometriosis can cause chronic pelvic pain ranging from 70%, the risk of ovarian tumors is 15-20%, the incidence of infertility is around 30-40%, the risk of turning malignant is 0.7-1% and psychological disorders. The incidence rate in Indonesia cannot be estimated because there are no epidemiological studies, but from hospital findings, the incidence ranges from 13.6-69.5% in the infertility group.[3]

Endometriosis is a benign gynecological disease; various factors have been hypothesized to play a role in developing endometriosis. This includes genetic and epigenetic profiles, inflammation, hormonal activity, menstrual cycle, prostaglandin metabolism, immunological factors and endocrine-disrupting chemicals (EDC).[4-6] Endocrine-disrupting chemicals (EDCs), such as phthalates and organochlorines, have estrogen-like properties. Exposure to EDC during intrauterine life will increase the risk of endometriosis in adulthood.[4] According to the revised American Society for Reproductive Medicine (r-ASRM), endometriosis is classified into 4 degrees, namely grade I (minimal), degree II (mild), degree III (moderate) and degree IV (severe).[7]

Several predictive factors are used to diagnose endometriosis non-invasively, including blood and urine biomarkers and endometrial features. However, the examination's accuracy level is still very low, around 56% -74%. Diagnosis by clinical examination and imaging (ultrasound, TVUS, and MRI) also has a low level of accuracy for several types of endometriosis, which are often found at laparoscopy. This is what causes delays in

the diagnosis of endometriosis. One of the other non-invasive examination methods that can be used to predict endometriosis is the measurement of the anogenital distance (AGD).[8,9]

ABG is a relatively new measurement parameter that indicates the distance from the anus to the genitalia. ABG measurements were performed using a ruler or calipers with millimeter accuracy, which was measured from the clitoris to the anus (anoclitoral distance/AGD-AC) and from the fourchette posterior to the anus (anofourchette distance/AGD-AF). Female fetuses have a shorter AGD than males; this is an early marker for determining sex during the first trimester. In males, ABG can be a marker of intrauterine androgen exposure. High intrauterine androgen exposure results in a longer AGD. In contrast, exposure to chemicals such as estrogen and some phthalates can reduce AGD.[10,11] A prospective cohort study conducted by Crestani A et al. showed a relationship between exposure to endocrine disruptors such as organochlorine chemicals and an increased risk of endometriosis.[4]

Based on the explanation above, this study aims to determine the relationship between ABG and the incidence of endometriosis in Dr. Wahidin Sudirohusodo General Hospital, Makassar, Indonesia.

## METHODS

This study was a cohort study using a cross-sectional study design using primary data on 70 samples to know the relationship between AGD and the incidence of endometriosis. This research was conducted at Dr. Wahidin Sudirohusodo and other educational network hospitals in Makassar. Sampling (AGD measurement) was conducted in the treatment and operating rooms. The research was conducted during the July 2022 period until the number of research samples was fulfilled using a purposive sampling technique. There are several inclusion criteria in this study, including 1) Willing to participate in research activities; 2) Women aged 20 - 40 years and not currently pregnant; 3) Unmarried or married but nullipara or multipara with a history of vaginal delivery; 4) There is no history of trauma to the perineum; 5) Not currently on hormonal treatment; 6) No history of endocrine disease; 7) normal body mass index; and 8) Women diagnosed with endometriosis from anamnesis, physical examination, and ultrasound, who will undergo laparoscopic/laparotomy surgery. The exclusion criteria in this study included 1) Patients with non-endometriosis intraoperative findings and 2) Non-endometriosis histopathological examination results.

Anogenital Distance (AGD) is the distance measured between the center of the anus and the posterior or anterior margin of the genitalia (AGD from the anus to the posterior fourchette is marked as AGD-AF, and the distance to the anterior clitoris is marked as AGD-AC) in units of mm. Endometriosis referred to in this study is the endometrium in an abnormal or ectopic location based on clinical symptoms, physical examination, supporting examinations, intraoperative findings, and histopathological examination results. Endometriosis is classified into stages I, II, III, and IV.

The data obtained was recorded and then analyzed using the Chi-Square method, a statistical method used to see the significance and relationship between unpaired categorical variables in a 2x2 table. The Chi-Square test requirements are cells with an expected value of less than 5, a maximum of 20% of the number of cells. If the Chi-Square test requirements are not met, the alternative test is Fisher's test. To clarify the relationship dynamics between risk and effect factors, look at the prevalence odds ratio (POR) value. For the interpretation of the results using a significance degree of α (P alpha) of 5% with a note that if p ≤0.05 (p-value ≤ p alpha), then H0 is rejected (there is a relationship between the independent and dependent variables), whereas if p≥0.05 then H0 is accepted (there is no relationship between the independent and dependent variables). If the data cannot be categorized, a correlation test will be carried out, but previously, a normality test will be carried out first using the Kolmogorov-Smirnov test; if the p-value is greater than 0.05 then the data is normally distributed and the next step will be using the Pearson test, but if the p-value is smaller than 0.05, the data is not normally distributed. The next step will use the Spearman test. Data were analyzed with SPSS version 22.0 for Windows.

## RESULTS

This study used 70 research subjects consisting of 35 endometriosis patients and 35 control patients, as seen in Table 1.

This study wanted to examine the ability of AGD-AF and AGD-AC as predictors of endometriosis, as shown in Table 2 and Table 3. Before that, it is necessary to carry out an analysis regarding the relationship between AGD-AF and AGD-AC related to the incidence of endometriosis, as well as a comparison with the non-endometriosis group.

Linear regression analysis used variables from the patient comparison table with a p-value ≤0.2. Linear regression analysis was used to determine the independent variables that could affect endometriosis predictors and to detect biases that could affect AGD-AF's ability to predict endometriosis. Variables that meet the requirements are AGD-AF scores, fertility, and menstrual cycles. There was no difference in AGD-AC length between the endometriosis and control groups, so further analysis was not carried out (Table 4).

Analysis of the difference in means and logistic regression has proven the ability of AGD-AF to detect endometriosis. Therefore, it is necessary to look for cut-offs and analyze the sensitivity and specificity of AGD-AF in detecting endometriosis. To be able to determine the cut-off value of AGD-AF, it is necessary to carry out an ROC analysis (Figure 1). The cut-off value can be determined from Youden's index. Based on Youden's index, the best cut-off value that can be used is 23.35 mm, with a sensitivity and specificity value for detecting endometriosis, respectively 97.1% and 65.7%.

## DISCUSSION

The first discussion is in Table 1, where the characteristics of the research subjects are listed. Endometriosis is a disease that causes frequent and chronic pain in 10% of women of reproductive age.[10-13] This is in accordance with the characteristics of

the sample population shown in Table 1, where the median age of the patients in this study was 29 years. The relationship between AGD and age was found in a study by Lee D et al., who observed shorter AGD in postmenopausal women.[14]

These findings differ from this study, which found no relationship between ABG length and age (p>0.05). This may be related to the age of the sample used, namely 20-40 years, which is the age before menopause. Regarding the menstrual cycle, there were no abnormal cycles in the endometriosis group, whereas in the non-endometriosis group,

**Table 1. Characteristics of the sample population**

| Variable | Endometriosis (n=35) | Non-endometriosis (n=35) | p |
|---|---|---|---|
| Age (Years) | 30.06±5.7 | 29.5±4.65 | 0.650[b] |
| Menstrual Cycle | | | |
| Normal | 29 (58.00%) | 21 (42.00%) | 0.034[a] |
| Abnormal | 0 (0.00%) | 6 (100.00%) | |
| Menstrual bleeding | | | |
| Metrorrhagia | 3 (33.33%) | 6 (66.67%) | 0.53[a] |
| Menorrhagia | 3 (60.00%) | 2 (40.00%) | |
| Age of Menarche (Years) | 13.62±1.05 | 13.74±1.09 | 0.658[b] |
| Menstrual Duration (Days) | 5.17±1.15 | 4.62±1.30 | 0.070[b] |
| Dysmenorrhea | | | |
| Yes | 32 (91.40%) | 3 (8.60%) | <0.01[a*] |
| No | 3 (8.60%) | 32 (91.40%) | |
| Marriage | 13 (48.14%) | 14 (51.86%) | 0.806[a] |
| Fertile | | | |
| Yes | 3 (27.27%) | 8 (72.73%) | 0.048[a] |
| No | 14 (70.00%) | 6 (30.00%) | |
| Infertility duration (Years) | 6.47±4.22 | 9.86±18.24 | 0.461[b] |
| Level of education | | | |
| Low education | 3 (30.00%) | 7 (70.00%) | 0.172[a] |
| High education | 32 (53.33%) | 28 (46.67%) | |

[a]Independent T-Test; [b]Chi-Square; *Statistically significant if p-value less than 0.05

**Table 2. Comparison of AGD-AF Values in Endometriosis and Non-endometriosis Groups**

| Independent Variable | AGD-AF | | p |
|---|---|---|---|
| | Endometriosis | Non-endometriosis | |
| AGD | 22.65 ± 6.54 | 29.53 ± 3.52 | <0.001* |
| Fertile | 33.00 ± 1.13 | 30.03 ± 3.99 | 0.191* |
| Infertile | 20.69 ± 4.12 | 27.70 ± 2.83 | <0.001* |
| Menstrual Cycle | | | |
| Normal | 22.34 ± 6.94 | 29.86 ± 3.23 | <0.001* |
| Abnormal | | 28.50 ± 1.4 | |
| Menstrual Bleeding | | | |
| Menorrhagia | 25 ± 6.27 | 26.85 ± 6.03 | 0.724 |
| Metrorrhagia | 23.33 ± 1.72 | 29.03 ± 4.88 | 0.298 |

*Statistically significant if p-value less than 0.2 and then included in the multivariate analysis

**Table 3. Comparison of AGD-AC Values in Endometriosis and Non-endometriosis Groups**

| Independent Variable | AGD-AC | | p |
|---|---|---|---|
| | Endometriosis | Non-endometriosis | |
| AGD | 68.04 ± 5.8 | 66.81 ± 5.49 | 0.369 |
| Fertile | 74.60 ± 3.53 | 66.1 ± 5.68 | 0.43 |
| Infertile | 65.53 ± 4.32 | 66.4 ± 7.78 | <0.001* |
| Menstrual Cycle | | | |
| Normal | 68.69 ± 6.04 | 65.61 ± 5.09 | 0.070 |
| Abnormal | | 66.67 ± 5.45 | |
| Menstrual Bleeding | | | |
| Menorrhagia | 64.80 ± 6.03 | 74.75 ± 3.46 | 0.202 |
| Metrorrhagia | 64.96 ± 2.20 | 68.76 ± 5.80 | 0.322 |

*Statistically significant if p-value less than 0.05

**Table 4. AGD-AF Multivariable Linear Regression**

| Variable | Multivariate | |
|---|---|---|
| | Coef β (95% CI) | p |
| AGD-AF | 0.526 | <0.001* |
| Fertile Patients | 0.245 | 0.039 |
| Infertile Patients | -0.148 | 0.196 |
| Normal Menstrual Cycle | 0.090 | 0.452 |

*Statistically significant if p-value less than 0.05

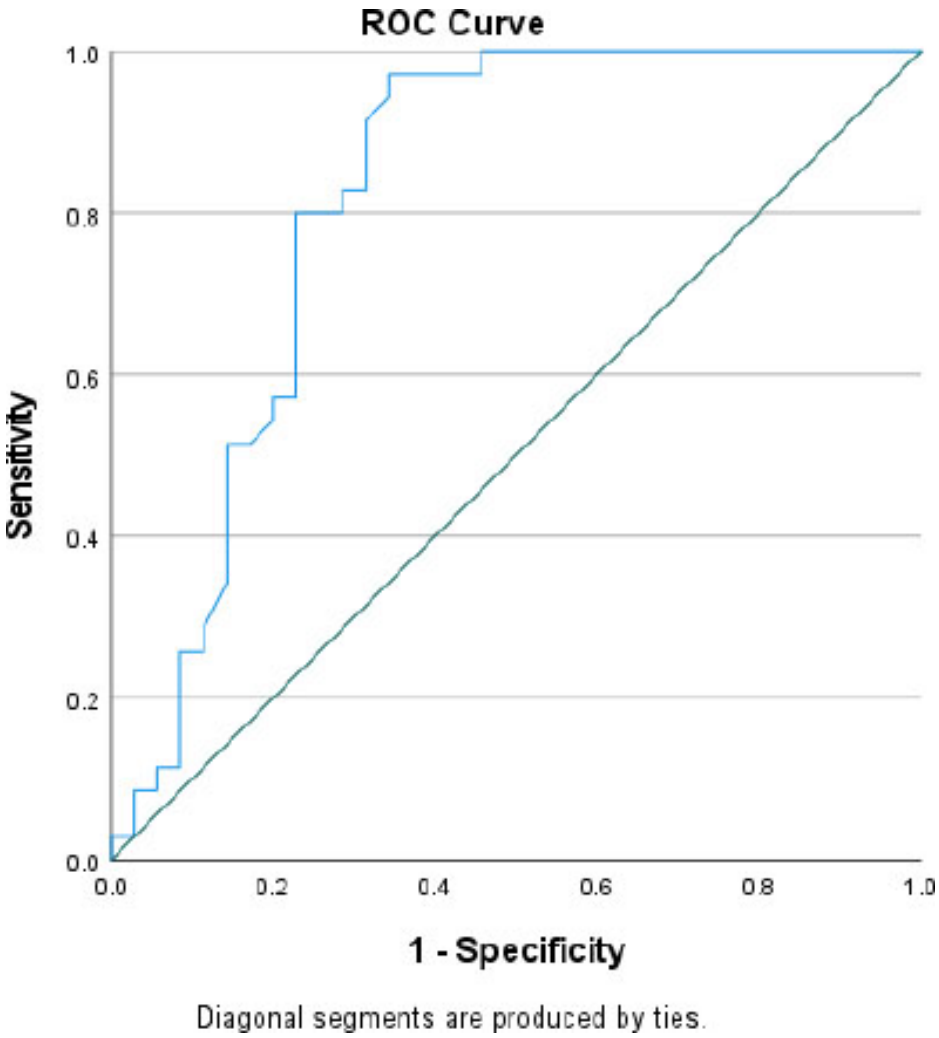


**Figure 1.** ROC curve AGD-AF length as a predictor of endometriosis.

17.1% experienced oligomenorrhea. This is not in accordance with the study of Wei M et al., which showed that in a meta-analysis study, the odds ratio for the incidence of endometriosis in patients with menstrual cycles of less than 27 days and more than 29 days was 1.22 and 0.68.[15] Endometriosis is an ectopic tissue that is responsive to sex hormones and causes a chronic inflammatory condition with cyclic painful manifestations, especially during menstruation.[10,16] This explains the condition of dysmenorrhea or pain during menstruation in endometriosis group patients who more were found compared to controls, namely 32 patients or 91.4% in the endometriosis group, compared to 3 patients or 8.6% in the non-endometriosis group. Nnoaham KE et al., in a meta-analysis study, showed that earlier menarche has a higher risk of endometriosis.[17] These findings differ from this study, which found no difference in menarche age in the endometriosis and non-endometriosis patient groups. This is probably due to the many factors that influence the incidence of endometriosis after puberty. Based on statistics in Table 1, the group of patients who were married and experienced infertility was more in the endometriosis group, compared to the non-endometriosis group, namely 40% compared to 17.1% respectively ($p \leq 0.05$). This is following the findings by Tomasetti and D'Hooghe et al., which showed the possibility of more frequent infertility in endometriosis patients.[12]

In the discussion in Table 2, where a comparison of AGD-AF values was carried out in the two study subject groups, it was found that there were significant differences in the length of AGD-AF in the endometriosis group compared to non-endometriosis, namely 22.65 ± 6.54 and 29.53 ± 3.52 ($p < 0.001$). This is in accordance with research by Crestani et al., showing a relationship between the length of AGD-AF and the incidence of endometriosis. With a cut-off of 20 mm, AGD-AF can be used to predict the incidence of endometriosis.[4] Furthermore, the comparison of infertile cases was also significant, namely 20.69 ± 4.12 in the endometriosis group and 27.70 ± 2.83 in the control group ($p < 0.001$). This finding is consistent with a study by Weinstock T et al., which found a relationship between AGD, age and fertility.[18]

Menstrual cycles obtained in all endometriosis groups were normal cycles, and the comparison of AGD-AF values was significant, namely 22.34 ± 6.94 compared to 29.86 ± 3.32 ($p \leq 0.001$) in the non-endometriosis group. The abnormal menstrual cycle was not found in the endometriosis group, so it could not be analyzed. This is different from the findings of previous studies, where it was found that patients with short menstrual cycles were associated with higher exposure to plasma estrogen than patients with normal and prolonged menstrual cycles.[19] For the last variable, namely the amount of bleeding and the length of menstruation, there was no significant difference in the AGD-AF values in the endometriosis and non-endometriosis groups. There has been no previous research regarding the relationship between ABG and the amount of bleeding or the length of menstruation.

In Table 3, a significant result is the AGD-AC comparison in the endometriosis

and non-endometriosis groups related to the incidence of infertility. This is in accordance with previous research by Wainstock T et al., which found a relationship between AGD, age and fertility.[19] For other variables, the AGD-AC ratio in the endometriosis and non-endometriosis groups was insignificant, so no further analysis was carried out for the AGD-AC values.

The linear regression analysis in Table 4 proved that only the AGD-AF length was a determinant for the incidence of endometriosis ($p \leq 0.001$) with a 95% CI. The AGD-AF ability bias was then confirmed by repeating linear regression using only AGD-AF as the independent variable in capital. In comparison of the B value in the analysis, AGD-AF proved not to change more than 10%, so it can be concluded that the variables of fertility, intertidal, and menstrual cycle are not variables that can interfere with the ability of AGD-AF as a predictor of endometriosis.

ABG length is a measurement that is considered easy to do to detect gender dimorphism in the early life of the fetus. In contrast to earlier research, which stated that the length of AGD in rat studies was relatively stable throughout life, some studies show differences in the length of AGD after exogenous and anti-androgen exposure.[20] Research by Crestani A et al. shows a relationship between the length of AGD-AF and the incidence of endometriosis. With a cut-off of 20 mm, AGD-AF can predict the incidence of endometriosis with a sensitivity and specificity of 30.6% and 98.6%.[4] This is consistent with the findings in this study, which found differences in AGD-AF length between endometriosis patient groups and controls. However, in Figure 1, the ROC curve of the AGD-AF length in this study has a different cut-off of 23.35 mm with a sensitivity and specificity value for detecting endometriosis, respectively 97.1% and 65.7%. The relationship between AGD length is also supported by research by Sánchez-Ferrer ML et al., which found shorter AGD-AF and AGD-AC in endometriosis patients compared to controls and groups of polycystic ovarian syndrome (PCOS) patients. This study proved AGD-AF is a better predictor than AGD-AC, with the sensitivity and specificity of AGD-AF in predicting endometriosis were 84% and 91.4% with a cut-off of 20.9 mm.[21]

Another independent variable we assessed in this study was the stage of endometriosis. Mendiola J et al. confirmed the relationship between ABG and the incidence of endometriosis and endometrioma. The length of AGD-AF in the group below the median of the study had an OR 41.6 times higher for deep-infiltrating endometriosis than the group with a length of AGD-AF above the median.[19] However, until now, no other studies have studied the relationship between the length of AGD and the stage of endometriosis. This study's findings illustrate no relationship between AGD-AF and AGD-AC on the endometriosis stage.

Ca-125 values are elevated in several conditions other than endometriosis. CA-125 increases in conditions with peritoneal disorders in the abdomen, so it is not a good marker for detecting endometriosis.[22] This study found no correlation between the length of AGD and CA-125 levels. In addition, ABG length was also not found to have a correlation with cyst size in endometriosis group patients. Until now, no research has studied the correlation between ABG length and CA-125 levels and endometriosis cyst volume.

## CONCLUSION

This study shows a significant relationship between the length of AGD and the incidence of endometriosis, especially AGD-AF, so it can be used as a predictor in assisting the diagnosis of endometriosis. Whereas for AGD-AC, there was no relationship with the incidence of endometriosis. This study has a higher AGD-AF cut-off than previous studies regarding the relationship between AGD-AF and endometriosis.

## CONFLICT OF INTEREST

The authors declare that there is no competing interest regarding the manuscript.

## ETHICAL CONSIDERATION

This research was conducted based on the ethical research from the Faculty of Medicine Ethics Committee, Universitas Hasanuddin, Makassar, Indonesia.

## FUNDING

The authors are responsible for the study's funding without involving a grant, scholarship, or other funding resource.

## AUTHOR CONTRIBUTION

All authors contributed to the study from the conceptual framework, data gathering, and analysis until the study's results were interpreted upon publication.

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