eISSN (Online): 2598-0580

Bioscientia Medicina: Journal of Biomedicine & Translational Research

Journal Homepage: www.bioscmed.com

Sensitivity and Specificity of Vascular Endothelial Growth Factor A (VEGF-A) against Endometriosis

Heriyadi Manan[1*], Edo Rezaldy Edward[2]

[1]Division of Fertility, Endocrinology and Reproduction, Department of Obstetrics and Gynecology, Faculty of Medicine, Universitas Sriwijaya, Palembang, Indonesia
[2]Subspecialized Residency Training, Division of Fertility, Endocrinology and Reproduction Department of Obstetrics and Gynecology, Faculty of Medicine, Universitas Sriwijaya, Palembang, Indonesia

ARTICLE INFO

Keywords:
Sensitivity
Specificity
Endometriosis
VEGF-A
Diagnostic test

***Corresponding author:**
Heriyadi Manan

E-mail address:
hydplg2@gmail.com

All authors have reviewed and approved the final version of the manuscript.

https://doi.org/10.32539/bsm.v5i7.384

ABSTRACT

Background. Endometriosis is a benign disorder defined by the presence of endometrial glands and stroma outside the uterus. Endometriosis occurs in 10-15% of women during their reproductive years. Angiogenesis and the inflammatory response are important factors in the development of endometriosis. The formation of a new blood supply is a crucial step in the formation of endometrial lesions. Angiogenesis is induced by a growth factor peptide, namely vascular endothelial growth factor A (VEGF-A). VEGF-A is known as a vascular permeability factor that plays an important role in the pathological angiogenesis process and is a more specific and prominent angiogenesis factor among the VEGF family. The purpose of this study was to determine the sensitivity and specificity of examination VEGF-A of menstrual blood in diagnosing endometriosis compared to laparoscopy. **Methods.** This diagnostic test research has been carried out at the gynecology polyclinic, especially the division of Fertility, Endocrinology and Reproduction, Faculty of Medicine, Universitas Sriwijaya – Dr. Mohammad Hoesin General Hospital from August to November 2018, there were 45 patients who met the inclusion criteria. VEGF-A examination in instrumental blood based on ELISA examination. Data analysis to measure sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy using Medcalc statistics. **Results.** From the results of this study, it is known that the majority of the study samples were aged 31.69 years (24-38 years) 75.6%, body mass index (BMI) 51.1% with normal BMI, experienced infertility by 82.2% of the sample, most of the samples did not smoke (95.6%) and most experienced mild pain 55.6% using the VAS pain scale. From this study, it is known that 84.4% or 38 of the study samples had endometriosis with a cut off point value of VEGF-A > 347 pg/mL. From the results of this study, it is known that the sensitivity of VEGF-A in diagnosing endometriosis is 84.2%, specificity is 85.7%, positive predictive value is 97%, negative predictive value is 50% and accuracy is 84%. **Conclusion.** VEGF-A menstrual blood can be used as a diagnostic tool for endometriosis.

1. Introduction

Endometriosis is a benign disorder defined by the presence of endometrial glands and stroma outside the uterus, associated with pelvic pain and infertility.[1] Endometriosis is a broad spectrum of symptoms and signs, tends to be progressive and recurs, causing symptoms and on the quality of life, ability to work and female fertility as well as problems with doctors.[2] Theory of implantation as described Sampson in 1921 still believed to be the beginning of endometriosis, involving backflow of menstrual blood, inflammation, implantation and growth of endometrial tissue in the peritoneum.

Endometriosis occurs in 10-15% of women of reproductive age with pelvic pain, dysmenorrhea,

dyspareunia and infertility[3] with a peak incidence at the age of 25 to 35 years.[4] The prevalence of endometriosis in the United States is 2-50%[5] and ranks third for hospitalization after pelvic infection and ovarian cysts.[6] A woman's risk of developing severe endometriosis is six times that of first-degree female offspring than in women who do not have endometriosis. The diagnosis of endometriosis can be delayed due to non-specific symptoms. On average, women take 7 to 11 years from the onset of symptoms to a definitive diagnosis of endometriosis. Laparoscopy is the gold standard in diagnosing endometriosis. The use of laparoscopy in diagnosing endometriosis is delayed due to cost factors, the ability of the surgeon and the long course of the disease with early treatment of symptoms using non-steroidal anti-inflammatory drugs, contraceptive pills.[7]

Many theories suggest the pathogenesis of endometriosis such as metaplasia theory, immune theory, hormone theory, environmental factors and the most popular is the retrograde menstrual theory, but the retrograde menstrual theory fails to explain from 90% of the backflow of menstrual blood to the female pelvic cavity, only 15% develop endometriosis. Thus angiogenesis and inflammatory response become important factors. Recent studies have demonstrated the important role of angiogenesis in the pathogenesis of endometriosis. The formation of a new blood supply is a crucial step in the formation of endometrial lesions. Angiogenesis is induced by a growth factor peptide, namely growth factor vascular endothelial A (VEGF-A).[8]

VEGF-A is an important growth factor for the initiation of sprouting angiogenesis and regulates capillary cell immigration, endothelial cell proliferation and gene expression in vascular morphogenesis. Transforming growth/actor (TGF) acts as a regulator of expression VEGF-A. Abnormal TGF-P signaling results in the growth of endometrial lesions and several studies have shown elevated TGF-P levels in the peritoneal fluid, peritoneum, and ectopic endometrial lesions of women with endometriosis.[1] VEGF-A as a patent angiogenic factor is known to be increased in the peritoneal fluid of women with endometriosis compared to those without endometriosis.levels VEGF-A are related to the stage of endometriosis originating from ectopic endometrial lesions and peritoneal macrophages. VEGF-A is known as a vascular permeability factor that plays an important role in the process of pathological angiogenesis. VEGF-A is a more specific and prominent angiogenesis factor among the VEGF(I) family.[2]

Menstrual blood provides an opportunity as a new diagnostic tool because elements of the endometrium are returned to the pelvic cavity during menstruation and make the diagnosis process simpler and faster. Study angiogenesis markers of endometrial from menstrual blood in women with and without endometriosis can help establish an earlier diagnosis.[9] This study is the study VEGF-A first menstrual blood in establishing the diagnosis of endometriosis.

2. Methods

This research is an analytical study with a diagnostic test design which was carried out in August 2018 until the number of samples was met.examination Direct visual of endometriosis in laparoscopic procedures for endometriosis indications in the operating room of Dr. Mohammad Hoesin Palembang, then the examination of the biological marker VEGF-A was carried out using the ELISA method at the Prodia Palembang Laboratory.

The sample in this study were women of reproductive age with complaints of dysmenorrhea, menstrual disorders and infertility who voluntarily wanted to participate in the study and met the inclusion criteria. Sampling was done by consecutive sampling with a minimum of 42 samples. Inclusion criteria in this study were subjects aged years, subjects with clinical symptoms of endometriosis (15-45dysmenorrhea, dyspareunia, dysuria, abnormal uterine bleeding), willing to participate in the study and signing informed consent. Exclusion criteria in this study were pregnant women, subjects who had been diagnosed with other causes such as malignancy, chronic inflammation, and diabetic retinopathy.

After the complete data was collected, laboratory examination of VEGF-A was performed, then data analysis was carried out to determine the sensitivity

and specificity of VEGF-A in menstrual blood having a good diagnostic value or not in diagnosing endometriosis.

3. Results

In this study, 45 samples were obtained consisting of 38 subjects with endometriosis and 7 subjects without endometriosis.

The sociodemographic distribution in this study is presented in Table 1. Of the 45 research samples, the majority in this study were aged 20-35 years, namely 34 subjects (75.6%). Most of the study subjects with normal BMI were 23 (51.1%). Most of the subjects did not smoke as many as 43 (95.6%). A total of 37 (82.2%) study samples experienced infertility. Most of the research subjects experienced mild pelvic pain as many as 25 (55.6%) study subjects.

Table 1. Sociodemographic distribution (n = 45)

Characteristics	n	%
Age		
20 – 35 years	34	75.6
> 35 years	11	24.4
Body Mass Index		
Underweight	2	4.4
Normal	23	51.1
Overweight	20	44.4
Smoking		
Yes	2	4.4
No	43	95.6
Infertility		
Yes	37	82.2
No	8	17.8
Pelvic		
No pain	9	20.0
Mild pain	25	55.6
Moderate pain	8	17.8
Severe pain	3	6.7 The

Distribution based on endometriosis is categorized into two, namely endometriosis and non-endometriosis which are presented in Table 2. Of the 45 study samples, the majority of the study subjects had endometriosis, as many as 38 (84.4%), while 7 (15.6%) subjects did not have endometriosis.

Table 2. Distribution by Endometriosis (n = 45)

Characteristics	n	%
Endometriosis	38	84.4
No Endometriosis	7	15.6
Total	45	100.0

The results of the analysis using the test Chy Square to determine the relationship between pelvic pain and VEGF-A are presented in Table 3. After the test Chy Square, The relationship between pelvic pain and VEGF-A showed that there was a significant relationship ($p < 0.05$) withvalue $p = 0.020$. Of the 45 study subjects, the subjects with VEGF-A majority of positive felt mild pelvic pain, namely 20 (44.4%) subjects, subjects with moderate pelvic pain were 7 (15.6%), while subjects who felt severe pelvic pain and

no pain were only 3 (6.7%) subjects. Of the 12 study subjects with VEGF-A negative, the majority felt no pelvic pain, namely 6 (13.3%) subjects, 5 (11.1%) subjects experienced mild pelvic pain, 1 (2.2%) subjects experienced moderate pelvic pain, and none of the subjects who experienced severe pelvic pain 0 (0%).

Table 3. Relationship of pelvic pain with VEGF-A (n = 45)

Pelvic Pain	***VEGF-A***				***p value***
	Positive		**Negative**		
	n	**%**	**n**	**%**	
No pain	3	6.7	6	13.3	0.020
Mild pain	20	44.4	5	11.1	
Moderate pain	7	15.6	1	2.2	
Severe pain	3	6.7	0	0	
Total	33	73.3	12	26.7	

In this study, the variable was VEGF-A categorized based on the, the results of the analysis showed that the value cut-off pointVEGF-A cut off point was > 347 so that the category was VEGF-A positive if the VEGF-A value was > 347.

Diagnostic test between VEGF -A The laparoscopy was examined using laparoscopy as the gold standard in diagnosing endometriosis, carried out on 45 research subjects, then analyzed using SPSS software version 19.0, Medcalc and Epicalc. The results of the analysis are presented in Table 4.

Table 4. Diagnostic test between VEGF-A against laparoscopy (n = 45)

VEGF-A	**Laparoscopic (Endometriosis)**		**Total**
	Positive	**Negative**	
	n	**n**	
Positive	32	1	33
Negative	6	6	12
Total	38	7	45

Measures of diagnostic test values consist of sensitivity, specificity, accuracy, positive likelihood ratio, negative likelihood ratio, positive predictive value, negative predictive value, likelihood ratio test, and AUC. Analysis of the results of examination VEGF-A with the gold standard of laparoscopy for diagnosing endometriosis using the ROC curve, the results are presented in Table 5. Table 5 shows that the diagnostic value of VEGF-A against laparoscopy (endometriosis) obtained sensitivity VRGF-A in diagnosing endometriosis 84.2%, specificity 85.7 %, positive predictive value 97, negative predictive value 50%, and accuracy 84%.

Table 5. Diagnostic value of VEGF-A on laparoscopy (n= 45)

Diagnostic Value of	***VEGF-A***	**95% *Confidence Interval***
Sensitivity	84.2%	(68 – 93)
Specificity	85.7%	(42 – 99)
PPV	97%	(82 – 100)
NPV	50%	(22 – 78)
PLR	5.89%	(0.96 – 36.38)
NLR	0.18%	(0.08 – 0.41)
LR test	32.72 %	
Accuracy	84%	(0.70 – 93)
AUC	0.853%	(0.716 – 0.941)

4. Discussion

This study shows the incidence of endometriosis occurred at a mean age of 31.69 years (32 years) of 75.6%. Age of women with endometriosis in Dr. Mohammad Hoesin was no different compared to the age of women with endometriosis in the research of Wahyu Hadisaputra, Sandhy Prayudhana, 2012 at Dr. RSUPN. Cipto Mangunkusumo Jakarta and Tita Madjid, Dedy Hendry, 2016 at RSUP Dr. Hasan Sadikin Bandung at the age of 34, 1 year and 34, 2 years. [10]

Body mass index (BMI) of the women with dismenorrhea complaints, menstrual disorders and infertility occurs with a mean BMI of 24.2 kg / m^2 to 51.1% in women with endometriosis with normal BMI. These results are not different from the research of Wahyu Hadisaputra, Sandhy Prayudhana, 2012 at Dr. RSUPN. Cipto Mangunkusumo Jakarta.[10]

Most of the women with endometriosis in this study did not have a smoking habit (95.6%). Some studies suggest that smoking will increase the level of cytokines hypoxia- induced VEGFin human endothelial cell cultures.[6]

Women in the sample of this study amounted to 82.2% had infertility problems. Several studies, such as the research of Macer, et al (2012) showed the causes of infertility in endometriosis have not been able to explain the mechanism well.[11,12] Empirically, the cause of infertility is caused by anatomical distortion of the internal genital organs, especially in the adnexa, namely the occlusion of the tube, and adherence to the surrounding organs (adherent).

Mild pain occurred in 55.6% of the study sample on the measurement of the VAS score. This is similar to the study of Fauconnier and Chapron (2005) which stated that the causal relationship between pain was related to the anatomic location of the endometrioma lesion, the effect of compression, or the infiltration of nerve fibers in the subperitoneal pelvic space by ectopic endometrial implants.[13] Thus, this study showed a significant relationship between pelvic pain and the incidence of endometriosis ($p = 0.020$).

The incidence of endometriosis in this study sample occurred in 38 people (84.4%) of 45 people during the study period who found endometriosis lesions during laparoscopy (gold standard) at Dr. Mohammad Hoesin Palembang. The incidence of this study is greater than the research by Wahyu Hadisaputra, Sandhy Prayudhana, 2012 at Dr. RSUPN. Cipto Mangunkusumo Jakarta (69.5%) with a minimum sample size of 38 people. [10]

However, the incidence of endometriosis in this study was higher than the prevalence of endometriosis in women of reproductive age in the United States (2 - 50%).

The Value marker VEGF-Awas higher in women with endometriosis compared to women without endometriosis which was statistically significant ($p < 0.05$). This level is similar to the research conducted by Dunselman, et al (2001), Jimenez, et al (2010), and Lu, Y, et al (2017) which showed higher levels of the VEGF-A marker in women with endometriosis. This confirms that retrograde menstruation carrying ectopic

endometrial cells neutrophils, macrophages, proinflammatory cytokines, patent angiogenic factor (VEGF-A) causes the acute and chronic inflammatory processes that form endometriosis.

The cut off point of the VEGF-A value in the menstrual blood of women with endometriosis in this study was 347 pg/mL with an inlay Area Under Curve (AUC) of 85.3 including good. This study has a different VEGF-A value from the research of Wahyu Hadisaputra, Sandhy Prayudhana, 2012 at Dr. RSUPN. Cipto Mangunkusumo Jakarta with cut off VEGF-A serumof 314.75 pg/mLY.[5]

Based on the research, there were 32 of 38 samples with values marker VEGF-A> 347 pg/mL (84.2%) and 6 of 7 samples with values marker VEGF-A< 347 pg/mL (85.7%) and statistically significant ($p < 0.05$). The results of the VEGF-A diagnostic test based on the Receiver Operating Characteristic (ROC) Curve on the incidence of endometriosis in women of reproductive age with complaints of dysmenorrhea and HIV disorders who came to the Gynecology Polyclinic, Dr. Mohammad Hoesin has a sensitivity of 84.2%, a specificity of 85.7%, a positive predictive value of 97%, a negative predictive value of 50%, and an accuracy of 84%.

Thus, this study shows that VEGF-A can be used as a marker goodin diagnosing the presence of ectopic endometrial lesions through menstrual blood so that it can help establish a diagnosis valid, reliable, and acceptable.

5. Conclusion

Based on the results of this study, it can be concluded that the results of the VEGF-A diagnostic test based on ROC on the incidence of endometriosis in women of reproductive age with complaints of dysmenorrhea, menstrual disorders, and endometriosis who came to the Gynecology Polyclinic, Dr. Mohammad Hoesin has a sensitivity of 84.2%, a specificity of 85.7%, a positive predictive value of 97%, a negative predictive value of 50%, and an accuracy of 84%.

6. References

1. Fritz, M, Speroff L. Endometriosis. In: Clinical Gynecologic Endocrinology and Infertility. 8th ed. Philadelphia: Lippincott Williams & Wilkins, a Wolters Kluwer business; 2011. p. 1221-48.
2. Hogg S, Vyas S. Endometriosis. Obstet Gynaecol Reprod Med 2015; 25(5): 133-41.
3. Mehedintu C, Plotogea MN, Ionescu S, Antonovici M. Endometriosis is still a challenge. J Med Life. 2014; 7(3): 349- 57.
4. Giudice LC. Endometriosis. N Engl J Med. 2010; 362(25): 2389-98.
5. Scarselli G, Rizzello F, Cammilli F, Ginocchini L, Coccia ME. Diagnosis and treatment of endometriosis. A review. Minerva Ginecol. 2005; 57(1): 55-78.
6. Yamamoto A, Johnstone EB, Bloom MS, Huddleston HG, Fujimoto VY. A higher prevalence of endometriosis among Asian women does not contribute to poorer IVF outcomes. J Assist Reprod Genet. 2017; 34(6): 765-74.
7. Fassbender A, Burney RO, 0 DF, D'Hooghe T, Giudice L. Update on Biomarkers for the Detection of Endometriosis. Vol. 2015, BioMed Research International. 2015.
8. Lu Y, Yi X, Chen L. Study on the validity of serum VEGF-A for the diagnosis of endometriosis. Am J Clin Exp Gynecol. 2017; 4(1): 20- 5.
9. Burney RO, Giudice LC. Pathogenesis and pathophysiology of endometriosis. Vol. 98, Fertility and Sterility. 2012. p. 511-9.
10. Wahyu Hadisaputra, Sandy Prayudhana. Serum biomarker profiles of interleukin-6 in endometriosis staging. Med J Indonesia. 2013.
11. Macer ML, Taylor HS. Endometriosis and Infertility. A Review of the Pathogenesis and Treatment of Endometriosis-associated Infertility. Obstetrics and Gynecology Clinics of North America. 2012.
12. Bulun SE. Endometriosis. Yen Jaffe's Reprod Endocrinol Physiol Pathophysiol Clin Manag Eighth Ed. 2018;609-642.e7.

13. Donnez J, Smoes P, Gillerot S, Casanas-Roux F, Nisolle M. VEGF in endometriosis. Hum Reprod. 1998;13(6):1686-90.