Research Article ISSN 2639-9342

Gynecology & Reproductive Health

A Comparison of Vascular Endothelial Growth Factors (Vegf) Levels in Menstrual Blood Between Women with and Without Endometriosis

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Received: 17 November 2020; **Accepted:** 11 December 2020

Citation: Andi Cakra, Nasrudin AM, Eddy Tiro, et al. A Comparison of Vascular Endothelial Growth Factors (Vegf) Levels in Menstrual Blood Between Women with and Without Endometriosis. Gynecol Reprod Health. 2020; 4(6): 1-5.

ABSTRACT

***Background**: Vascular endothelial growth factor (VEGF) is one of the main stimuli in endometriosis angiogenesis. This study aims to compare VEGF levels in menstrual blood between women with and without endometriosis. This research was carried out in dr. Wahidin Sudirohusodo General Hospital and several hospitals in collaboration with the Department of Obstetrics and Gynecology, Faculty of Medicine, Hasanuddin University, Makassar.*

***Method**: The method used was observational with a cross-sectional study design on 27 women with endometriosis and 17 women without endometriosis as a control group in February - July 2017. VEGF levels in menstrual blood were measured by the ELISA method.*

***Results & Discussion:** Data were analysed using statistical analysis by chi-square test and Mann-Whitney test. The results showed that VEGF levels in women with endometriosis were significantly higher than those without endometriosis (22,080.93 ± 9,216.42 pg/ml vs. 8,526.65 ± 2,981.38 pg/ml, $p<0.05$).*

***Conclusion**: We concluded that there was an increase in VEGF levels of menstrual blood in patients with endometriosis compared with those without endometriosis. These VEGF levels correlate positively with the stages of endometriosis. VEGF in menstrual blood can be used as biomarker and predictor examination panel to diagnose endometriosis which is minimally invasive.*

Keywords
Endometriosis, Menstrual blood, VEGF level.

Introduction

Endometriosis is a disease characterised by the presence of endometrial-like tissue outside the uterine cavity that causes chronic inflammation in the surrounding tissue. Endometriosis-related complaints include pelvic pain, dysmenorrhea, infertility, low back pain, dyspareunia, constipation, dysuria and diarrhea in which dysmenorrhea and infertility are the most common complaints in endometriosis patients. Accurate diagnosis methods besides laparoscopy or laparotomy and anatomical pathologic evidence have not yet been found [1].

The overall prevalence of endometriosis was estimated at 5-10% and the prevalence in infertile women itself was reported to be

38% (around 20-40%) [2]. This increases dramatically to as high as 25–50% in women with infertility and 30–50% of women with endometriosis have infertility [3] and in women with endometriosis who experience pain, infertility or both are between 25-35% [4].

The diagnosis of endometriosis based on symptoms is not reliable. Although the relationship between endometriosis and symptoms such as dysmenorrhea, non-menstrual pelvic pain, dyspareunia and infertility is widely accepted [5]; the predictive value of these symptoms is limited [4].

CA-125, cytokines and angiogenic and growth factors are differentially expressed in the peripheral blood of women with endometriosis compared with controls. None of the biomarker panels were validated as non-invasive tests for endometriosis [6], probably because most studies have limited number of patients and assessments of different cycle phases and stages of endometriosis [6].

Research on biomarker panels was also limited with respect to the number of biomarkers analysed, statistics use (univariate statistical analysis) and lack of validation in a series of tests for each patient [7].

Menstrual blood contains cells or tissues from the functional layer of the endometrium. This layer is formed after the end of menstruation. The proliferative phase of this menstrual cycle is induced by estrogen and progesterone from corpus luteum is increased (secretory phase). The absence of progesterone causes constriction of the arteries that supply blood to the functional layer so that the cells in this layer are ischemic, die and causes menstruation [8].

Angiogenesis is the main process that supports the development of endometriotic lesions. Vascular endothelial growth factor (VEGF) as one of the cell growth factors plays an important role in endothelial cell proliferation, angiogenesis, vasculogenesis and ovarian capillary hyperpermability in both physiologically and pathologically. In the ovarian cycle, VEGF is involved in follicular growth, ovulation, development of the corpus luteum and ovarian steroidogenesis.

Previous research by Vodolazkaia et al. [9] showed that plasma VEGF levels were elevated in women with mild and moderate endometriosis compared to women without endometriosis during the menstrual cycle. Menstrual blood has the potential as a non-invasive diagnostic marker for endometriosis. The development of non-invasive diagnostic tests for endometriosis will be of great value in clinical practice because it is possible to identify women with sub-fertility with or without pain as endometriosis patients.

Based on the background above, we were interested in conducting a study aimed at comparing VEGF levels in menstrual blood between women with and without endometriosis.

Methods

Location, Time of Research

The study was conducted from February to July 2017 at the Public Service Board of dr. Wahidin Sudirohusodo Hospital and several hospitals in collaboration with the Department of Obstetrics and Gynecology, Faculty of Medicine, Hasanuddin University, Makassar.

Study Design and Variables

The study design was observational with cross sectional study. The research variables consisted of: independent variables (VEGF levels in menstrual blood), dependent variable (endometriosis), intermediate variable (angiogenesis), and control variables (wound healing, inflammatory tissues).

Population and Sample

The study population was patients who underwent laparoscopy or laparotomy and were diagnosed with endometriosis. The research sample was the study population that met the inclusion and exclusion criteria.

Method of Collecting Data

Patient data were obtained using a questionnaire followed by menstrual blood collection and measurement of VEGF levels in menstrual blood.

Data Analysis Technique

Data were analyzed using Chi-square and Mann-Whitney test with a significance level of $p < 0.0.5$ and all data analysis results were presented in table form and processed with the SPSS program.

Result

An observational with cross sectional study was conducted to compare VEGF levels in menstrual blood between women with and without endometriosis from February to July 2017 at the Public Service Board of dr. Wahidin Sudirohusodo Hospital and several hospitals in collaboration with the Department of Obstetrics and Gynecology, Faculty of Medicine, Hasanuddin University, Makassar.

At the beginning of sampling, a total sample of 70 people met the inclusion criteria, consisting of 35 women diagnosed with endometriosis (n = 35) and 35 women without endometriosis (n = 35) as control of the study. Diagnosis is carried out by laparoscopy/laparotomy. When measuring VEGF levels, 8 menstrual blood samples from women with endometriosis and 18 from controls had lysis so that the total sample of 17 non-endometriosis patients grouped as controls (n = 17) and 27 with endometriosis (n = 27) consisting of 13 cases of mild endometriosis (stage I and II) and 14 were severe endometriosis (stage III and IV).

Most of the women with endometriosis group and the controls were in reproductive age (≤ 35 years old). Compared to the control group, women with endometriosis had almost the same proportion between nulliparity and multiparity, and experienced pain during

menstruation (dysmenorrhea) (Table 1).

VEGF measurement results in 44 samples showed significantly different levels of VEGF ($p < 0.05$) based on parity and infertility (Table 2).

Characteristics	Endometriosis (n=27)		Control (n=17)	
	n	%	n	%
Age (years old)				
≤35	21	77.8	15	88.2
>35	6	22.2	2	11.8
Stages				
I/II (Mild)	13	48.1	0	0
III/IV (Severe)	14	51.9	0	0
Education (years)				
<9	7	25.9	8	47.1
≥9	20	74.1	9	52.9
Work				
Yes	11	40.7	6	35.3
No	16	59.3	11	64.7
Parity				
Nulliparity	14	51.9	0	0.0
Multiparity	13	48.1	17	100.0
Infertility				
Yes	12	44.4	0	0.0
No	15	55.6	17	100.0
Dysmenorrhea				
Yes	19	70.4	0	0.0
No	8	29.6	17	100.0

Table 1: Characteristics of the Samples.

Variable	VEGF				Total		P Value	OR (CI 95%)
	High		Low					
	n	%	n	%	N	%		
Dysmenorrhea								
Yes	10	52.6	9	47.4	19	100.0	0.052	4.444 (1.174 -16.820)
No	5	20.0	20	80.0	25	100.0		
Infertility								
Yes	8	66.7	4	33.3	12	100.0	0.011	7.143 (1.652 – 30.877)
No	7	21.9	25	78.1	32	100.0		
Age								
≤ 35 years old	12	33.3	24	66.7	36	100.0	1.000	0.833 (0.170 – 4.088)
≥ 35 years old	3	37.5	5	62.5	8	100.0		
Parity								
Nulliparity	8	57.1	6	42.9	14	100.0	0.042	4.381 (1.130 – 16.985)
Multiparity	7	23.3	23	76.7	30	100.0		
Stages								
I/II (mild)	1	7.7	12	92.3	13	100.0	0	(cannot be calculated)
III/IV (severe)	14	100.0	0	0.0	14	100.0		
Total	15	34.1	29	65.9	44	100.0		

Tabel 2: VEGF Levels based on Clinical Characteristics of the Samples.
p = Chi-square test analysis result

Comparison of VEGF levels is based on the stage of endometriosis. The results of the analysis with Mann-Whitney test showed that there were differences between VEGF levels of menstrual blood in severe endometriosis (III/IV) compared to mild endometriosis (I/II) indicating significantly higher VEGF levels in severe endometriosis (III/IV) (29,195.86 pg/ml vs. 14,418.69 pg/ml; $p < 0.05$) (Table 3). It also showed the differences between VEGF levels in the menstrual blood of endometriosis patients ($p < 0.001$) compared to the control group in which VEGF levels were significantly higher in endometriosis (22,080.93 ± 9,216.42 pg/ml vs. 8,526.65 ± 2,981.38 pg/ml; $p < 0.05$) (Table 4).

Stages	n	VEGF levels in menstrual blood (Mean ± SD pg/ml)	p
I/II (mild)	13	14418.69 ± 1599,43	<0.001
III/IV (severe)	14	29195.86 ±7368,68	

Table 3: VEGF Levels in Menstrual Blood according to the Stages of Mild Endometriosis (I/II) and Severe Endometriosis (III/IV).

p= Mann-Whitney test analysis result

Case Groups	VEGF levels (mean ± SD pg/ml)	*p*
Endometriosis	22080.93±9216.42	<0.001
Control	8526.65±2981.38	

Table 4: VEGF Levels in Menstrual Blood between Endometriosis and Control Group.

p = Mann-Whitney test analysis result

Discussion

This study shows an increase in VEGF levels in the menstrual blood of endometriosis patients. These findings can be interpreted that the development of endometriosis lesions also depends on the bioavailability of VEGF pathway activity in endometrial support tissues, measurement of VEGF levels carried out in menstrual blood samples of women with endometriosis.

The majority of the sample age is ≤ 35 years old and 77.8% of the women in the endometriosis group were aged ≤ 35 years. Similar results were shown by Abdullah [10] and Manuaba [11] studies, finding that the mean age in women with endometriosis is 32–34 years old. Endometriosis affects 10% of women in reproductive age. Clinical symptoms of dysmenorrhea, dyspareunia and pelvic floor pain worsen women's health. Study by Carvalho [12] proved an increased in cases of infertility caused by endometriosis. The incidence of endometriosis increases in reproductive age, especially at the age of 21-40, and decreases with women experiencing menopause at the age of 41-50 years [13].

The average age to diagnose endometriosis is between 25-30 years old [14]. This is consistent with the study report that showed the growth of endometriotic implants depends on the production of steroids by the ovary so that endometriosis affects most women at the age of 25-35 years [3].

In this study, 70.4% patients with endometriosis were experiencing dysmenorrhea. This result is similar to the result of a study by Ragab et al. [15] involving 654 young adult women with endometriosis and had a result of 48.9% (n = 320) who with dysmenorrhea in various severity of endometriosis; 68.8% of samples were with severe dysmenorrhea (n = 220/320).

The results of the analysis with Mann-Whitney test showed that there were differences between VEGF levels of menstrual blood in severe endometriosis (III/IV) compared to mild endometriosis (I/II) indicating significantly higher VEGF levels in severe endometriosis (III/IV) (29,195.86 pg/ml vs. 14,418.69 pg/ml; $p < 0.05$). In our study, there was a deviant standard deviation in the mild endometriosis group because of the second respondent that was not included in the range because of an extreme data with VEGF level of 18,101 pg/ml. In a further search of this patient, in addition to mild stage endometriosis, uterine myoma was found which did not undergo intraoperative myoma enucleation, so this contributed to an increase in VEGF level in this patient.

Our study showed that there were significant differences in the VEGF levels of menstrual blood in endometriosis group and control group ($p < 0.05$). Comparison of VEGF serum levels in endometriosis and control group had been measured by study of Abdullah [10] where there is an increase in VEGF levels according to the severity of endometriosis stage while low VEGF levels are found in patients without endometriosis. Women with endometriosis in Makassar showed increased VEGF levels in advanced stages of endometriosis and VEGF levels are lower in women without endometriosis compared with those who with endometriosis. The mean VEGF levels in the study were significantly higher than controls (334.18 pg/ml vs. 91.36 pg/ml; $p<0.05$). Nasrudin [16] conducted a study on the relationship between an angiogenic factor and endometriosis that is the level and expression of TGF-β1 in an increase according to the stage of endometriosis.

During the normal menstrual cycle, somatic cells are in the functional area of the endometrium to carry out programmed cell death which is a biological response that reduces the risk of cell survival and ectopic growth in the form of apoptosis causing the peritoneal cavity in retrograde menstrual flow. Women without endometriosis have monocytic type of macrophages in peritoneal fluid. On the contrary, women with endometriosis have more peritoneal macrophages. Hyperactive cells hide multiple growth factors and cytokines that enhance the development of endometriosis. The intact mesothelium membrane prevents the attachment of menstrual endometrial shale to the peritoneal membrane, thus preventing the development of endometriosis. It has not been agreed whether the peritoneum has been damaged regularly over a period of a month so that endometriosis can develop over time with a potent Fallopian tube.

Recent research showed that even though the ability of immune cells to identify and destroy autologous endometrial tissues is found in women with endometriosis, these cells retain their ability to produce cytokines, growth factors (IL-1, IL-6, IL-8, TNF, RANTES, VEGF) and strong angiogenic factors that ultimately support ectopic survival and growth from endometrial tissue fragments [3,17]. There are differences in peripheral and peritoneal expression and function patterns (both in ectopic fluid and lesions). The lymphocyte population in endometriosis patients is a result of disturbances in macrophages and natural killer cells functions, which originate at the genetic level. This suggests that increased VEGF levels in menstrual blood in patients with endometriosis may be an event caused by congenital differences in endometrial, peritoneal and systemic factors, causing abnormal peritoneal responses to menstrual debris and facilitating entopic implantation.

The main role of VEGF at the beginning of the event and the sustainability of endometriosis is associated with the Sampson hypothesis and the immunological approach to retrograde menstruation which causes the implantation of endometriotic tissues outside the uterine cavity where retrograde menstruation is reflux of menstrual bleeding through the Fallopian tubes and then implanted in the pelvic cavity, especially the peritoneum, and as a result of highly complex and interrelated immunological processes that contribute to the further growth of the endometrial cells that are released so that the cells persist, grow and develop in the peritoneum and adhesion to the peritoneum.

Menstrual blood has not been adequately explored in scientific research that focuses on the pathogenesis of endometriosis. Based on the existing literature and corroborated by the results of our study that there is a local contribution to the endometrial environment for the development of endometriosis, a permissive environment in the peritoneal cavity has also been shown to exist, allowing separate endometrial cells to be embedded and grow. This confirms the presence of inflammatory markers and angiogenesis in the menstrual blood of women with endometriosis and the controls. Macrophage and leukocyte activity significantly greater in menstrual blood than in peripheral blood in women with endometriosis reflects an increase in local inflammatory activity.

The limitation of our study is that many samples were analyzed because most of the samples were taken at far-flung locations from the laboratory where they were examined which took a time that exceeded the sample transfer deadline.

Conclusion

We concluded that there was an increase in VEGF levels of menstrual blood in patients with endometriosis compared with those without endometriosis. These VEGF levels correlate positively with the stages of endometriosis. We suggest that VEGF in menstrual blood can be used as a biomarker examination panel to diagnose endometriosis that is minimally invasive. Menstrual blood sampling should be done in a laboratory that can measure VEGF levels in menstrual blood directly to avoid the number of samples dropping due to lysis.

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