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by Mgs. Irsan Saleh17

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Research Article

Serum Vascular Endothelial Growth Factor (VEGF) in DMPA Acceptors: Influence on Bleeding Occurrence

Kadar Vascular Endothelial Growth Factor (VEGF) Serum pada Akseptor DMPA: Pengaruhnya pada Kejadian Perdarahan

Ratih Pratiwi[1], Rizani Amran[1], Usman Said[1], Irsan Saleh[2]

[1]*Department of Obstetrics and Gynecology*
[2]*Medicine and Health Research Unit*
Faculty of Medicine University of Sriwijaya/
Dr. Mohammad Hoesin General Hospital
Palembang

Abstract

Objective: To analyze the relationship between levels of serum vascular endothelial growth factor (VEGF) and bleeding occurrence in depo medroxyprogesterone acetate (DMPA) acceptors.

Method: We employed a cross-sectional study on 70 DMPA acceptors with DMPA use of 3 to 6 months who presented for midwifery service in Palembang. Blood samples were obtained in order to assess levels of serum VEGF using ELISA (enzyme-linked immunoabsorbent assay) method. Laboratory assessments were carried out in PRODIA laboratory in Jakarta.

Result: We recruited 70 subjects into our study. After 3 to 6 months of using DMPA, as much as 26 subjects (37.1%) reported complaints of bleeding and 44 subjects (62.9%) reported no bleeding. The mean level of serum VEGF in DMPA acceptors with bleeding was 355 ∓ 170 pg/ml, and 323 ∓ 202 pg/ml in acceptors with no bleeding. We identified a significant association between duration of use and bleeding occurrence ($p<0.05$). There was no significant difference in terms of serum VEGF levels between DMPA acceptors who experienced bleeding and those who did not ($p>0.05$).

Conclusion: In our sample, we found an association between duration of DMPA use and presence of bleeding but VEGF levels was not found to be different in women experiencing abnormal uterine bleeding and those who did not.

[Indones J Obstet Gynecol 2015; 1: 26-31]

Keywords: bleeding, DMPA acceptor, serum VEGF

Abstrak

***Tujuan**: Untuk menganalisa hubungan antara kadar VEGF serum pada akseptor DMPA dengan kejadian perdarahan di Kota Palembang.*

***Metode**: Kami melakukan penelitian potong lintang pada 70 akseptor DMPA dengan lama pemakaian 3 sampai 6 bulan yang datang ke praktek bidan di Kota Palembang. Dilakukan pengambilan sampel darah untuk diperiksakan kadar serum VEGF dengan menggunakan metode ELISA. Pemeriksaan laboratorium dilakukan di laboratorium PRODIA Jakarta.*

***Hasil**: Kami merekrut subjek penelitian sebanyak 70 orang. Setelah pemakaian DMPA 3 sampai 6 bulan, sebanyak 26 subjek melaporkan mengalami perdarahan (37,1%) dan 44 subjek (62,9%) tidak melaporkan perdarahan. Rerata kadar VEGF serum pada akseptor DMPA yang mengalami perdarahan adalah 355 ∓ 170 pg/ml, dan kelompok yang tidak mengalami perdarahan adalah 323 ∓ 202 pg/ml. Didapatkan hubungan yang signifikan antara lama pemakaian DMPA dan kejadian perdarahan ($p<0,05$). Tidak ditemukan perbedaan kadar VEGF serum yang bermakna antara kelompok dengan kejadian perdarahan dan kelompok yang tidak mengalami perdarahan ($p>0,05$).*

***Kesimpulan**: Pada sampel kami, terdapat hubungan antara lama penggunaan DMPA dengan kejadian perdarahan uterin abnormal namun tidak ditemukan perbedaan kadar VEGF serum antara perempuan yang mengalami perdarahan uterin abnormal dan yang tidak.*

[Maj Obstet Ginekol Indones 2015; 1: 26-31]

***Kata kunci**: akseptor DMPA, perdarahan, VEGF serum*

Correspondence: Ratih Pratiwi. Department of Obstetrics and Gynecology, Faculty of Medicine University of Sriwijaya. Palembang
Telephone: 08127318231. Email: dr.ratih79@yahoo.com

INTRODUCTION

Menstrual disorders are the most frequent complaint in women of reproductive age, which may include shortened or elongated menstrual cycles, irregularities in the quantity of bleeding, irregular bleeding or spotting, or even absence of periods (amenorrhea). In acceptors of the three-month injectable depo medroxyprogesterone acetate (DMPA), also known as Depo Provera, the most common complaint is a menstruation disorder, either in the form of spotting, amenorrhea or irregular menstruation. Nearly 40% of acceptors reported experiencing menstrual disorders by the end of the first injection of DMPA. In Palembang, injectable contraceptives is the most commonly used method of contraception in 2010, which makes up about 67.27% of all contraceptive use.[1,2]

The biochemical process explaining the cause of bleeding disorder in DMPA acceptors is still unconfirmed, although it is clearly known that the progression involves menstrual cycle regulatory mechanisms, which are associated with hemostasis, angiogenesis and endometrial bleeding.[3,4]

DMPA acceptors experience endometrial changes in the form of thickness and blood vessels integrity. The blood vessel walls become thin and fragile. An increase in progesterone receptor, decrease in estrogen receptor, and increase in vascular endothelial growth factor (VEGF) was also found. VEGF is a protein that stimulates growth, cell multiplication and maintenance of blood vessels. Changes in the normal distribution of VEGF caused by production of synthetic-steroid-induced VEGF may be responsible for the disruption in menstrual cycle.[5,6]

A study conducted by Charnock-Jones et al in 2000, measured the levels of VEGF in Implanon users through endometrial biopsy, and obtained a significant increase in VEGF levels. Another study using samples from endometrial biopsy, showed increased levels of VEGF in progestin users.[7,8] Beside in stromal and epithelial tissue, VEGF is also produced by leukocytes and platelets. However, most studies on VEGF involve taking samples from endometrial biopsy, and there is no data providing information on VEGF levels in the serum of DMPA acceptors. This is a pilot study on serum VEGF levels and the expected serum VEGF profiles in DMPA acceptors who experienced and did not experience abnormal uterine bleeding.

METHODS

This study is an observational analytic study using cross sectional method. The study is conducted in private midwife practices providing family planning services, especially using DMPA, from April 2013 to January 2014.

The population is DMPA acceptors aged 20 to 45 years old, with 3 to 6 months duration of use, not currently undergoing hormone treatment, and not suffering from any systemic disease. The types of abnormal uterine bleeding included in this study are metrorrhagia (irregular menstrual bleeding with increased frequency), menorrhagia (excessive menstrual bleeding occurring over several consecutive cycles), and menometrorrhagia (excessive menstruation occurring with increased frequency). Patients were asked to fill in a study sheet. Patient characteristics were obtained from the study sheet, and blood samples were obtained by staff of Prodia laboratory Palembang. Assessment of serum VEGF levels was performed in Jakarta, using ELISA method.

All statistical analyses were performed using SPSS version 22.0 for Windows. Data analysis includes descriptive statistics and inferential statistics. Descriptive statistics were used to display the data characteristics, which in this study is displayed in the form of frequency, mean, standard deviation (SD) and 95% confidence interval (95% CI). Inferential statistics was analyzed by using chi-square, Fisher's exact test, and t-test, as appropriate, with a significance value of $p<0.05$.

RESULTS

The number of samples who met the inclusion criteria and participated in this study was 80 subjects. After the blood samples were evaluated, one sample could not be used due to lysis. After assessing VEGF levels (Quantikine, ELISA), 9 subjects did not complete the study sheet causing them to be excluded from the study. The final number of subjects analyzed in this study was 70 subjects.

Subject characteristics of our study sample are presented in Table 1. The majority of our samples were aged 20 to 34 years old (53 subjects, 75.7%) while 17 subjects (24.3%) were aged 35 to 40 years old. In terms of parity, most of our subjects (38.6%) have had two deliveries. As much as 60% of our subjects graduated from high school. We can also see that almost all of our subjects (90%) had a period duration of 4-7 days. After using DMPA for 3 to 6 months, most women do not experience abnormal uterine bleeding (62.9%), while those complaining of abnormal uterine bleeding (metror-

rhagia, menorrhagia, menometrorrhagia) amounted to 26 subjects (37.1%). Almost 90% of our subjects have had 2 DMPA injections which equals to 6 months of DMPA use. Furthermore, women with normal body mass index (BMI) in our study comprised the most common group comsisting of 46 subjects (65.7%).

Table 1. Subject Characteristics (n = 70)

Characteristics	Frequency	
	n	%
Age (year)		
20 - 34	53	75.7
35 - 40	17	24.3
Parity		
1	22	31.4
2	27	38.6
≥ 3	21	30.0
Education		
Junior high school	24	34.3
High school	42	60.0
University graduate	4	5.7
Duration of menstrual period (day)		
1 - 3	2	2.9
4 - 7	63	90.0
≥ 7	5	7.1
Abnormal uterine bleeding		
Present	26	37.1
Absent	44	62.9
Duration of DMPA use		
3 months (1 injection)	8	11.4
6 months (2 injections)	62	88.6
BMI		
Underweight	12	17.1
Normal	46	65.7
Overweight	10	14.3
Obese	2	2.9

Serum VEGF levels of our subjects are presented in Table 2. Based on our results, the lowest serum VEGF level in women who experience bleeding after DMPA use was 96.8 pg/ml, while the highest was 813.1 pg/ml. Whereas in women who did not experience bleeding after DMPA use, VEGF levels ranged from 79.7 pg/ml to 859.5 pg/ml. The mean VEGF concentration was higher in women who reported bleeding than those who did not.

Table 2. Serum VEGF Level (n = 70)

Serum VEGF (pg/ml)	Abnormal Uterine Bleeding	
	Present	Absent
Mean ∓ SD	355 ∓ 170	323 ∓ 202
Lowest	96.8	79.7
Highest	813.1	859.5

We performed bivariate analysis on the patient characteristics and VEGF levels and its correlation with presence of abnormal uterine bleeding. The results are presented in Table 3. We discovered that out of the patient characteristics, only duration of DMPA use was significantly correlated to the occurrence of bleeding, while age, parity, duration of menstrual period, and BMI was not significantly correlated to the presence of abnormal uterine bleeding. Moreover, between women who reported bleeding and those who did not, no significant difference was detected in the serum VEGF levels (p=0.4).

DISCUSSION

This study is a cross sectional study aimed to determine the neovascularization profile causing the occurrence of abnormal uterine bleeding in acceptors of 3-month injectable contraceptives. DMPA is a type of progestin contraceptive widely used by Indonesian women, administered via intramuscular injection every 12 weeks. Its mechanism of action is by inhibiting of ovulation, thickening the cervical mucus and affecting endometrial receptivity.

Table 3. Bivariate Analysis

Characteristic	Bleeding		No bleeding		p
	n	%	n	%	
Age (year)					
20 - 34	21	30.0	32	45.7	0.4*
35 - 40	5	7.1	12	17.1	
Parity					
1	10	14.3	12	17.1	
2	8	11.4	19	27.1	0.5*
≥ 3	8	11.4	13	18.6	
Duration of menstrual period (days)					
1 - 3	1	1.4	1	1.4	
4 - 7	23	32.9	40	57.1	1†
≥ 7	2	2.9	3	4.3	
Duration of DMPA use					
3 months (1 injection)	7	10.0	1	1.4	0.003†
6 months (2 injections)	19	27.1	43	61.4	
BMI					
Underweight	3	4.3	9	12.9	
Normal	20	28.6	26	37.1	0.5†
Overweight	3	4.3	7	10.0	
Obese	0	0	2	2.9	
Serum VEGF level (pg/ml)					
Mean ∓ SD	355 ∓ 170		323 ∓ 202		0.4‡

* *Chi-square*
† *Fisher exact test*
‡ *Unpaired t*

Nonetheless, acceptors of the method commonly report abnormal uterine bleeding. The form of abnormal bleeding in DMPA contraceptive users is unpredictable, with most acceptors experiencing long periods of bleeding or spotting. The menstrual disorder occurring in users of DMPA may even be amenorrhea (12% in the first 3 months of use, 46% after 1 year of use and 80% after 5 years or more). The abnormal uterine bleeding is rarely severe, but is a common cause of discontinuation. Approximately 20-25% of DMPA acceptors will discontinue use in the first year due to the occurrence of bleeding or menstrual disorder.[2,9,10]

The cause of abnormal bleeding in DMPA acceptors is still unconfirmed. Based on previous studies, the cause is suspected to be chronic endometritis or atrophy. Bleeding will generally decrease with time and longer usage. This bleeding may also be caused by continuous endometrial exposure to progesterone, which will lead to less exposure of endometrium to estrogen. This will in turn cause histopathological changes in the endometrium, which by not undergoing secretion phase eventually became thin.[3,11-13]

In endometrial progestin contraceptive users vascular changes occur in the endometrium. An increase in endometrial microvascular density, thinning of the walls of superficial endometrial blood vessels, changes in the basement membrane and increased vascular fragility has been discovered.

Endometrial tissue of progestin contraceptive users typically becomes atrophic and thin. Angiogenesis activity in endometrial progestin contraceptive users is lower than those not using progestin. This is supported by studies showing lower endometrial endothelial cell proliferation in progestin contraceptive users compared to women not using progestin contraceptives.[7,14,15]

The cause of increased vascular fragility is not clearly understood. The discovery of irregular changes in the endometrial surface, suggests the presence of abnormal angiogenesis with neovascularization on the endometrial surface. Formed blood vessels have a wider diameter and thinner walls. These blood vessels are also more fragile and prone to bleeding with disruption of blood flow and decreased blood vessel elasticity. Findings have shown a decrease in pericytes and abnormal basal membrane composition. Therefore, the evidence suggests a disruption in endometrial molecular and cellular functions in women with continuous exposure to progesterone, leading to the process of angiogenesis, increased tissue destruction, and hindered healing process by increased expression of matrix metalloproteinases, endothelial dysfunction, VEGF production, decrease in epithelial cytokeratin expression, increased expression of tissue factor, changes in the concentration and migration of endometrial leukocyte, as well as changes in other angiogenic factors.[7,14-16]

VEGF is a potent angiogenic factor produced by endometrial glandular and stromal cells as well as the myometrium. VEGF is upregulated in response to estradiol, estriol, and progesterone exposure. Changes in the normal distribution of VEGF induced by synthetic steroids may cause abnormal uterine bleeding. This condition can be caused by increased vascular permeability, changes in the balance of thrombotic factors or increased capillaries.[5,17-19]

In this study, the average VEGF levels in acceptors who experienced bleeding (metrorrhagia, menorrhagia, menometrorrhagia) was 355∓170 pg/ml, with the lowest level being 96.80 pg/ml, and the highest being 813.10 pg/ml. While in acceptors who did not report abnormal uterine bleeding, the average level of VEGF was 323∓202 pg/ml, with levels ranging from 79.70 pg/ml to 859.50 pg/ml. After statistical analysis using independent samples t-test, the p value was found to be 0.49. Thus, it was concluded that serum VEGF levels in DMPA acceptors does not affect the presence of abnormal uterine bleeding.

According to Fasciani et al, VEGF is associated with several benign disorders of the female reproductive tract, such as endometrial hyperplasia, abnormal uterine bleeding, and ovarian cysts. Meanwhile, according to Jelkmann, measurement of circulating VEGF levels can be used as diagnostic and prognostic value in cardiovascular disorders, inflammatory diseases, and malignancies. Normal levels of serum VEGF quantified using ELISA based on the Quantikine kit used is 62-707 pg/ml. In a study by Gosselin et al determining VEGF levels in the serum of endometriosis patients, the average level of VEGF in the control group was 221∓128 pg/ml. Cooper et al studied preoperative serum VEGF levels in ovarian cancer, and determined 246 pg/ml as the cutoff for distinguishing malignant and benign mass. Furthermore, it has been noted that preoperative VEGF level >380 pg/ml is a risk factor for death from malignant ovarian cancer.[20-22]

Based on our bivariate statistical test, a significant correlation was only identified between duration of DMPA use and presence of abnormal uterine bleeding. In studies examining endometrial histology in DMPA users, the incidence of abnormal bleeding decreased with duration of usage, but no relationship was identified between endometrial histology with complaints of bleeding. Another study in Swedish women using DMPA continuously for 36 months identified the presence endometrial atrophy through biopsy. Patterns of endometrial bleeding resulting from exposure to progesterone-containing contraceptives will vary according to the progestogen used, the dose, duration, and endogenous estrogen and progesterone levels, which is different in each individual. Results of research conducted by Pamuji et al demonstrated that with increasing duration of DMPA use, bleeding pattern will change from irregular bleeding and spotting to amenorrhea.[23,24]

Gosselin et al found elevated levels of VEGF to be associated with BMI. The mechanism behind this association is still unconfirmed. Mora and Pessin, in previous observations, suggested that adipose cells have the ability to produce VEGF.[21,25] However, our study found no significant association between serum VEGF levels and BMI (p=0.5).

CONCLUSION

There is no significant difference in serum VEGF levels between DMPA acceptors who experienced abnormal uterine bleeding and those who did not. Further research needs to be conducted with a larger sample size and longer observation time. Plasma VEGF quantification is also recommended to determine the relationship between VEGF levels with incidence of abnormal uterine bleeding in DMPA acceptors.

REFERENCES

1. BKKBN. Data Statistik KB Provinsi Sumatera Selatan tahun 2006-2010. Palembang: Biro Pusat Statistik Sumatera Selatan; 2012.
2. Golberg AB, Grimes DA. Injectable contraceptives. In: Hatcher RA, Trussel J, Nelson AL, et al. (Eds.) Contraceptive Technology. 19th ed. Sheffield: Ardent Media Inc; 2008: 61-2.
3. Thurman AR, Soper DE. Endometrial histology of depomedroxy progesterone acetate users: a pilot study. Infect Dis Obstet Gynecol 2006: 1-6.
4. Mark L, Ian SF. Mechanism of abnormal uterine bleeding. Hum Reprod Update 2002; 8: 60-7.
5. Charnock-Jones DS, Macpherson AM, Archer DF, et al. The effect of progestin on vascular endothelial growth factor, estrogen receptor and progesterone receptor immunoreactivity and endothelial cell density in human endometrium. Hum Reprod 2000; 15(Suppl 3): 85-95.
6. The ESHRE Capri Workshop Group. Endometrial bleeding. Hum Reprod Update 2007; 13: 421-31.
7. Hickey M, Fraser IS. Surface vascularization and endometrial appearance in women with implants. Implication for the mechanisms of breakthrough bleeding. Hum Reprod 2002; 17: 2428-34.
8. Critchley HO. Endometrial morphology and progestogens. Ernst Schering Res Found Workshop 2005: 55-88.
9. Weston G, Rogers P. Endometrial angiogenesis. Bailliere's Clin Obstet Gynecol 2000; 14: 919-36.
10. Meduri G, Bausero P, Perrot-Applanat M. Expression of vascular endothelial growth factor receptors in the human endometrium: modulation during the menstrual cycle. Biol Reprod 2000; 62: 439-47.
11. Albrecht ED, Pepe GJ. Steroid hormone regulation of angiogenesis in the primate endometrium. Front Biosci 2003; 8: 416-29.
12. Gargett CE, Rogers PAW. Human endometrial angiogenesis. J Reprod Fertil 2001; 121: 181-6.
13. Maas JWM, Groothuis PG, Dunselman GAJ, et al. Endometrial angiogenesis throughout the human menstrual cycle. Hum Reprod 2001; 16(8): 1557-61.
14. Moller B. Human endometrial angiogenesis. An immunohistochemical study of the endometrial expression of angiogenic growth factors and their corresponding receptors. Uppsala: Acta Universitatis Upsaliensis; 2004.
15. Lockwood CJ, Schatz F, Krikun G. Angiogenic factors and the endometrium following long term progestin only contraception. Histol Histopathol 2004; 19: 167-72.
16. Critchley H, Krikun G, Schatz F, et al. Abnormal uterine bleeding during progestin-only contraception may result from free radical-induced alterations in angiopoietin expression. Am J Pathol 2002; 161(3): 979-85.
17. Roxana MP, Juan CI, Amato JG, et al. Hypoxia and cAMP stimulate vascular endothelial growth factor (VEGF) in human endometrial stromal cells: potential relevance to menstruation and endometrial regeneration. J Clin Endocrinol Metabol 1999; 84: 2245-8.
18. Hoeben A, Landuyt B, Highley MS, et al. Vascular endothelial growth factor and angiogenesis. Pharmacol Reviews 2004; 56(4): 549-80.
19. Smith SK. Angiogenesis, vascular endothelial growth factor and the endometrium. Hum Reprod Update 1998; 4(5): 509-19.
20. Gagne D, Page M, Robitaille G, et al. Levels of vascular endothelial growth factor (VEGF) in serum of patients with endometriosis. Hum Reprod 2003; 18(8): 1674-80.
21. Jelkmann W. Pitfalls in the measurement of circulating vascular endothelial growth factor. Clin Chemist 2001; 47(4): 617-23.
22. Cooper BC. Ritchie JM, Broghammer CLW, et al. Preoperative serum vascular endothelial growth factor levels: significance in ovarian cancer. Clin Cancer Res 2002; 8: 3193-7.
23. Hickey M, Fraser I. Human uterine vascular structures in normal and diseased states. Micros Res Technique 2003; 60(4): 377-89.
24. Pamuji ES, Basuki D, Hakimi M. Association between bleeding pattern and estradiol level of depot medroxy progesterone acetat users. Berita Kedokteran Masyarakat 2008; 24(2): 51-7.
25. Mora S, Pessin JE. An adipocentric view of signaling and intracellular trafficking. Diabetes Metab Res Rev 2002; 18: 345-56.

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