Am J Clin Exp Obstet Gynecol 2017;4(1):20-25
www.ajceog.us /ISSN:2330-1899/AJCE0G0030027

Original Article

Study on the validity of serum VEGF-A for diagnosis of endometriosis

Yuemei Lu[1,2,3*], Xin Yi[1,2,3*], Liping Chen[1,2,3]

[1]Department of Obstetrics and Gynecology, The People's Hospital of Haian, Haian, Jiangsu, China; [2]Department of Anatomy and Neurobiology, Nantong University, Nantong, Jiangsu, China; [3]Department of Obstetrics and Gynecology, The First People's Hospital of Nantong, Nantong, Jiangsu, China. [*]Equal contributors.

Received April 7, 2016; Accepted August 6, 2016; Epub January 15, 2017; Published January 30, 2017

Abstract: Recent studies showed that VEGF-A involved in both the etiology and maintenance of endometriosis. This study was performed on women with and without endometriosis to evaluate the role of serum level of VEGF-A in comparison to CA-125 in diagnosis and follow-up of patients with endometriosis. The level of VEGF-A protein in eutopic and ectopic endometrium of the patients was assessed by Western blot. Serum level of VEGF-A and CA-125 were detected by ELISA kit. We found that VEGF-A protein expression was higher in ectopic endometrium than in eutopic endometrium and higher in endometriosis grade III-IV than in endometriosis grade I-II ($P<0.05$). The serum level of VEGF-A and CA-125 in patients before surgery was higher than after surgery and those without endometriosis. Rate of decline of VEGF-A level after surgery was higher than that of CA-125 in patients with endometriosis grade III-IV ($P<0.05$). The validity of VEGF-A for diagnosis of endometriosis grade I-II was better than that of CA-125. This study demonstrated that VEGF-A was associated with endometriosis and the expression of VEGF-A protein has a relationship with the severity of endometriosis. The serum level of VEGF-A is a better noninvasive and inexpensive way to earlier diagnosis of the endometriosis and to follow-up the patients with endometriosis grade III-IV.

Keywords: Endometriosis, VEGF-A, CA-125

Introduction

Endometriosis is defined as the proliferation of endometrial tissue outside the uterine cavity which can cause infertility, pelvic pain, and dysmenorrhea. It is one of the most common chronic and benign gynecologic disorders, affecting 5~10% of women in reproductive age. Endometriosis commonly develops possibly because of large quantities of backwashed menstrual tissue implanting on pelvic organs, mainly on the pelvic peritoneum and ovaries [1]. Although many theories have been suggested for the pathogenesis of endometriosis, it is believed that is a polygenically inherited disease with a complex, multifactorial etiology and is related to complex interaction of genetic, immunologic, hormonal, and environment factors. The most popular pathogenesis theory, transport theory, fails to explain why not all women with retrograde flow of endometrial tissue during menstruation develop endometriosis, but angiogenesis and inflammatory response are thought to be important [2].

Recent study shows that angiogenesis play a key role in the pathogenesis of endometriosis. Establishment of a new blood supply is crucial for the development of endometriotic lesions. Angiogenesis is induced by various peptide growth factors, including VEGF, basic fibroblast growth factor (bFGF) and thymidine phosphorylase [3]. VEGF is the most widely studied in endometriosis among various angiogenic factors. VEGF is a heparin-binding glycoprotein which includes VEGF-A, -B, -C, -D, -E and placental growth factor (PlGF). VEGF-A known as vascular permeability factor is considered to play a central role in both physiological and pathological angiogenesis. It is the most specific and prominent angiogenic factor among all VEGF family members. Recent studies showed that VEGF-A was involved in both the etiology and maintenance of peritoneal endometriosis [4]. Many studies focused on its involvement in the serum and peritoneal fluid of the endometriosis patients and showed patients with endometriosis compared with healthy women had higher VEGF levels in the serum and peritoneal fluid

Table 1. Characteristics of patients with and without endometriosis

	Age (years)	BMI (kg/m^2)
Control group (n=20)	35.50±7.44	21.40±2.84
Grade I-II group (n=18)	35.17±8.31	21.61±3.05
Grade III-IV group (n=22)	35.95±6.34	22.36±2.92
F-value	0.057	0.629
P-value	0.945	0.537

[5-7]. The diagnosis of endometriosis is difficult and delayed because of its non-specific symptoms and late presentation. The use of laparoscopy for diagnosis of endometriosis is limited by available funding, the surgeon's experience and its invasive nature. Diagnostic test of endometriosis has mostly concentrated on the levels of cancer antigen-125 (CA-125) and cytokines. However, none of them showed both high sensitivity and specificity in the diagnosis and follow-up of endometriosis.

The aim of this study was to evaluate the role of serum level of VEGF-A in comparison to CA-125 in diagnosis and follow-up of patients with endometriosis after surgery.

Materials and methods

Ethics

This study was approved by the institutional review board of the People's Hospital of Haian. Written informed consent for the use of tissue was obtained from each woman involved in the study.

Patients

A retrospective analysis was conducted on women with and without endometriosis in the proliferative and secretory phases of the menstrual cycle from May 2011 to October 2013 at Obstetrics and Gynecology Department, the People's Hospital of Haian, China. 40 patients with a surgery and histological diagnosis of endometriosis were selected. The stage of endometriosis was determined according to the classification of the American Society for Reproductive Medicine (ASRM) [8]. 20 women of reproductive age subjected to surgery for simple ovarian cyst removal and surgery confirmation of the absence of endometriotic lesions were selected as the control group. All women enrolled in the study fulfilled the following criteria: ① no hormonal treatment received 3 months prior to surgery; ② regular menses (defined by the presence of cycles lasting between 28 and 30 days in the previous year, with less than 5 days of flow); ③ patients without other gynecological pathologies, autoimmune diseases and cardiovascular diseases; ④ no previous pelvic surgery. There was not statistically significant difference regarding age and body mass index (BMI) (P>0.05) (**Table 1**).

The expression of VEGF-A protein was assessed by Western blot

Ectopic endometrium was prepared from patients with endometriosis who underwent laparoscopic surgery for primary treatment and diagnosis was confirmed by a clinician pathologist. Eutopic endometrial samples were obtained from all cases prior to laparoscopy using an endometrial biopsy curette. The samples were flushed with phosphate-buffered saline (PBS) and normal saline (NS) to remove contaminating blood then stored at -80°C until use.

The above mentioned samples were washed in ice-cold PBS and suspended in a protein extraction reagent (Pierce, Rockford, Illinois). Aliquots of total protein (50 μg per lane) were electrophoresed on a 12% SDS-polyacrylamide gradient gel and transferred to nitrocellulose membranes (Millipore). Washed in rinse buffer at RT and incubated in blocking buffer (5% fat-free milk in rinse buffer) for 30 min, the membranes were incubated for 4 h at RT with anti-human VEGF-A antibody (Santa Cruz Biotechnology, California; 1:500 dilution). Further washed with rinse buffer, the membranes were incubated with 1:800 diluted Alexa Fluor® 594-conjugated secondary antibody (Santa Cruz) for 2 h at RT. β-actin was used as a reference protein. The optical densities were analyzed by using Image Master TM2D Platinum (Version 5.0, Amersham Biosciences, Piscataway, NJ). Protein band intensity was quantified using Quantity One Analysis software (Bio-Rad Laboratories, Los Angeles, California).

Serum level of VEGF-A and CA-125 were detected by ELISA kit

Blood (serum) samples were collected in the morning before surgery from all cases. Postoperatively, in the follicular phase of the third

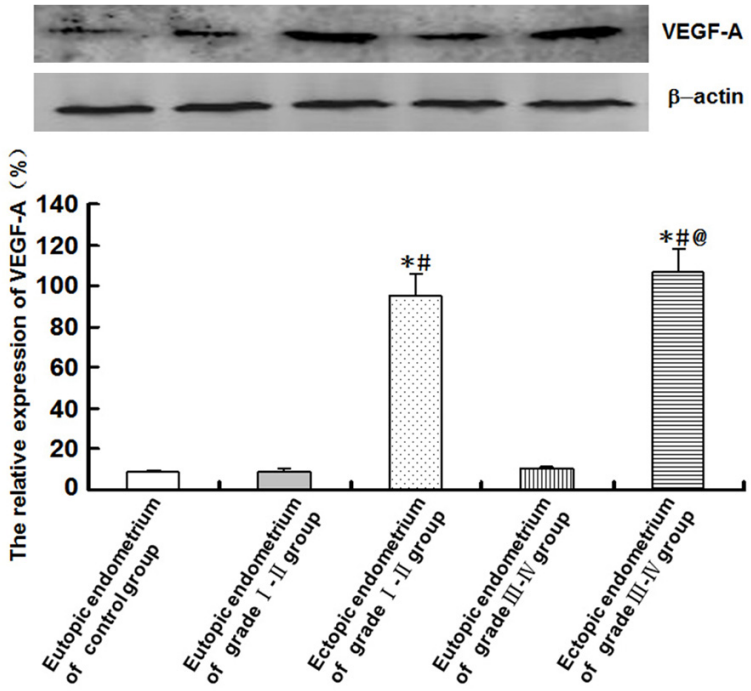

Figure 1. The expression of VEGF-A protein in endometrial tissues. The expression of VEGF-A protein in endometrial tissues of patients was measured by Western blot. Compared with the eutopic endometrium of the women in the control group and patients' own eutopic endometrium, the VEGF-A protein expression in the ectopic endometrium of patients with endometriosis increased. The VEGF-A protein expression in the ectopic endometrium of endometriosis grade III-IV was higher than that of endometriosis grade I-II. **P*<0.05, VS. Eutopic endometrium of control group Control group; #*P*<0.05, VS. Eutopic endometrium of e grade I-II and III-IV group; @P<0.05, VS. ectopic endometrium of grade I-II group.

menstrual cycle, blood samples were collected from 40 patients with endometriosis. 5 ml venous blood was withdrawn into nonheparinised tubes and kept at room temperature (RT) for 30 min. Then blood samples were centrifuged for 15 min at 3000 rpm and serum was obtained and stored at -80°C until use. VEGF level was measured by Human VEGF Quantikine ELISA Kit (Yanyu Biological Technology Co, Shanghai, China). CA-125 levels were detcted by using ELISA kit for CA-125 (Fangcheng Biotechnology Co., Ltd., Beijing, China).

Statistical analysis

Statistical analysis was performed using statistics package for social science 21.0 (SPSS 21.0). Nonparametric quantitative data were expressed as mean ± SD and compared using T-test or one-way analysis of variance (ANOVA). Qualitative data were expressed as percentages and compared using χ^2 or fisher exact test. *P*<0.05 was considered statistically significant.

Results

The level of VEGF-A protein in the endometrial tissue

Compared with the eutopic endometrium of the women in the control group and patients' own eutopic endometrium, the VEGF-A protein expression in the ectopic endometrium of patients with endometriosis increased and the difference between them was statistical significance (*P*< 0.05). The VEGF-A protein expression in the ectopic endometrium of endometriosis grade III-IV was higher than that of endometriosis grade I-II and the difference was statistical significance (*P*<0.05) (**Figure 1**).

Serum level of VEGF-A and CA-125

The serum level of VEGF-A and CA-125 in patients with endometriosis before surgery was higher than that of women without endometriosis and the difference was statistical significance (*P*<0.05). The serum level of VEGF-A and CA-125 in patients with endometriosis after surgery decreased and almost was still higher than that of women without endometriosis and the difference was statistical significance (*P*<0.05) (**Table 2**).

The rate of decline of VEGF-A level after surgery was similar to that of CA-125 in patients with endometriosis grade I-II (*P*>0.05). The rate of decline of VEGF-A level after surgery was higher than that of CA-125 in patients with endometriosis grade III-IV and the difference was statistical significance (*P*<0.05) (**Table 3**).

The validity of VEGF-A and CA-125 for diagnosis of endometriosis

The sensitivity, specificity and accuracy of VEGF-A for diagnosis of endometriosis grade I-II was 88.88%, 95.00%, 92.11% respectively.

Table 2. The serum level of VEGF-A and CA-125 in patients

	Control group (n=20)	Grade I-II group (n=18)		Grade III-IV group (n=22)	
		Before surgery	After surgery	Before surgery	After surgery
VEGF-A (pg/ml)	115.50±78.24	700.57±111.72*	367.92±32.71*,#	738.35±78.74*	368.43±35.22*,#
CA-125 (μg/ml)	15.30±6.00	27.67±12.70*	12.58±2.25#	38.59±4.72*	24.03±4.42*,#

*P<0.05, VS. Control group; #P<0.05, VS. Before surgery.

Table 3. The rate of decline of serum VEGF-A and CA-125 level in patients (%)

	Grade I-II group (n=18)	Grade III-IV group (n=22)
VEGF-A	45.42±14.44	49.04±11.30
CA-125	44.21±26.25	36.90±12.99
t-value	0.170	3.308
P-value	0.866	0.002

Table 4. The validity of VEGF-A and CA-125 for diagnosis of endometriosis grade I-II

	Cut-off	Sensitivity	Specificity	Accuracy
VEGF-A	440 pg/ml	88.88%	95.00%	92.11%
CA-125	35 μg/ml	55.56%	95.00%	76.32%
χ^2-value		4.985	0.000	3.563
P-value		0.026	1.000	0.059

Table 5. The validity of VEGF-A and CA-125 for diagnosis of endometriosis grade III-IV

	Cut-off	Sensitivity	Specificity	Accuracy
VEGF-A	440 pg/ml	95.45%	95.00%	95.24%
CA-125	35 μg/ml	90.90%*	95.00%	92.86%
χ^2-value		0.358	0.000	0.213
P-value		0.550	1.000	0.645

The sensitivity, specificity and accuracy of CA-125 for diagnosis of endometriosis grade I-II was 55.56%, 95.00%, 76.32%. The sensitivity and accuracy of VEGF-A for diagnosis of endometriosis grade I-II were higher than that of CA-125 and the difference between sensitivity was statistical significance (P<0.05) (**Table 4**).

The sensitivity, specificity and accuracy of VEGF-A for diagnosis of endometriosis grade III-IV was 95.45%, 95.00%, 95.24%. The sensitivity, specificity and accuracy of CA-125 for diagnosis of endometriosis grade III-IV was 90.90%, 95.00%, 92.86%. The sensitivity and accuracy of VEGF-A for diagnosis of endometriosis grade III-IV were higher than that of CA-125 but the difference between them was no statistical significance (P>0.05) (**Table 5**).

Discussion

Endometriosis is a common chronic and benign disease that shares several characteristics with invasive cancer. Its pathogenesis is still not clear. The diagnosis of endometriosis is difficult and delayed because of its non-specific symptoms and late presentation. The use of laparoscopy for diagnosis of endometriosis is limited by available funding, the surgeon's experience and its invasive nature. The positive predictive value for suspected lesions at surgery vs. histological diagnosis of endometriosis lesions is only 45%. In addition, magnetic resonance imaging (MRI) and ultrasound imaging not reliably identifies endometriosis lesions. Diagnostic test of endometriosis has mostly concentrated on the levels of CA-125 and cytokines. CA-125, a high molecular weight glycoprotein (20,000 Da), is well established as a diagnostic marker for endometriosis [9]. However, compared laparoscopy, serum CA125 levels has no value as a diagnostic tool. CA-125 is elevated in other diseases such as uterine leiomyomata, pelvic inflammatory disease and ovarian cancer, which potentially reduce its specificity. Moreover, some studies showed CA-125 only was accurate at diagnosing women with later stages of endometriosis because that circulating levels of CA-125 of women with minimal endometriosis is not higher than that of women without endometriosis [10, 11]. In addition, some investigators reported that CA-125 levels fluctuated during the menstrual cycle [12]. As a result, there is a need for development of noninvasive diagnostic tests for this condition.

Recent studies showed that angiogenesis was an important pathological process in the pathogenesis of endometriosis. In angiogenesis, VEGF-A is a key mediator that can stimulates endothelial cell proliferation and migration and

increases vascular permeability. Many studies showed patients with endometriosis had higher VEGF levels in the serum and peritoneal fluid than that of healthy women [5-7, 13, 14]. So in this study, We investigated whether elevated serum VEGF levels are correlated with the stage of the disease. Such correlation would suggest a new noninvasive and inexpensive way to earlier diagnosis of the endometriosis.

This study showed the VEGF-A protein expression in the ectopic endometrium increased compared with the eutopic endometrium and the difference was statistical significance ($P<0.05$). The VEGF-A protein expression in the ectopic endometrium of endometriosis grade III-IV was higher than that of endometriosis grade I-II and the difference was statistical significance ($P<0.05$). VEGF-A production is stimulated by growth factors, hormones, cytokines and hypoxia. Increase of VEGF in ectopic endometrium may be due to immune system alterations which is an important role in the development of endometriosis. Activated immune cells and endometriotic implants in the peritoneal cavity of endometriosis patients produce amounts of cytokines, growth factors and angiogenic substances [15]. Moreover, high Estradiol concentrations in endometriotic lesions is found [16]. Estrogen is a potent stimulus of angiogenesis through a direct increase of VEGF expression [17]. Our data suggested that VEGF-A was associated with endometriosis and the expression of VEGF-A has a relationship with the severity of endometriosis. The higher the grade of endomertriosis, the more highly expressed of VEGF-A.

Our study showed that the serum level of VEGF-A and CA-125 in patients with endometriosis before surgery was higher than that of women without endometriosis and the difference was statistical significance. The results runs in agreement with previous stuies [4, 7, 18, 19]. In contrast, some studies showed there was no significant differences in serum level of VEGF-A between women with and without endometriosis. The researchers concluded that diagnosis of endometriosis by laboratory testing serum level of VEGF-A is not possible [20, 21]. However, the methodology of collection and processing of samples should be standardised and declared when serum is used for the measurement of VEGF-A in these studies. In fact, VEGF is stored in the alpha granules of circulating resting platelets and released during clotting. So spinning the samples for different times and with different forces may influence the levels of VEGF-A, in particular when samples are processed after more than two hours from collection [22, 23]. We believe that the methodology used in the study was not adequate to support their conclusion.

We found that the serum level of VEGF-A and CA-125 in patients with endometriosis after surgery decreased significantly and there was a highly significant decrease in VEGF-A level (45.9%) compared to that of CA-125 (25.8%) in the patients with endometriosis grade III-IV. The results indicated that serum level of VEGF-A maybe a better marker to follow-up the patients with endometriosis grade III-IV after surgery. This results runs in agreement with previous studies [4].

On comparing the validity of VEGF-A or CA-125 in diagnosis for endometriosis, we found the sensitivity and accuracy of VEGF-A for diagnosis of endometriosis grade I-II were higher than that of CA-125 and the difference between sensitivity was statistical significance. The sensitivity and accuracy of VEGF-A for diagnosis of endometriosis grade III-IV were higher than that of CA-125 but the difference between them was no statistical significance. The results indicated that the serum level of VEGF-A maybe a better marker for diagnosis for endometriosis grade I-II.

In conclusion, this study demonstrated that VEGF-A was associated with endometriosis and the expression of VEGF-A protein has a relationship with the severity of endometriosis. The serum level of VEGF-A is a better noninvasive and inexpensive way to earlier diagnosis of the endometriosis and to follow-up the patients with endometriosis grade III-IV.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Liping Chen, Department of Obstetrics and Gynecology, The First People's Hospital of Nantong, 6 Haierxiang Road, Nantong 226001, Jiangsu Province, China. Tel: +86 513 85061309; E-mail: yixinntu@foxmail.com

References

[1] Rock JA, Zacur HA, Dlugi AM, Jones HW Jr, Te-Linde RW. Pregnancy success following surgical correction of imperforate hymen and complete transverse vaginal septum. Obstet Gynecol 1982; 59: 448-51.

[2] Vinatier D, Dufour P, Oosterlynck D. Immunological aspects of endometriosis. Hum Reprod Update 1996; 2: 371-84.

[3] Folkman J, Shing Y. Angiogenesis. J Biol Chem 1992; 267: 10931-4.

[4] Mohamed ML, El Behery MM, Mansour SA. Comparative study between VEGF-A and CA-125 in diagnosis and follow-up of advanced endometriosis after conservative laparoscopic surgery. Arch Gynecol Obstet 2013; 287: 77-82.

[5] Bourlev V, Iljasova N, Adamyan L, Larsson A, Olovsson M. Signs of reduced angiogenic activity after surgical removal of deeply infiltrating endometriosis. Fertil Steril 2010; 94: 52-7.

[6] Cho SH, Oh YJ, Nam A, Kim HY, Park JH, Kim JH, Park KH, Cho DJ, Lee BS. Evaluation of serum and urinary angiogenic factors in patients with endometriosis. Am J Reprod Immunol 2007; 58: 497-504.

[7] Xavier P, Belo L, Beires J, Rebelo I, Martinez-de-Oliveira J, Lunet N, Barros H. Serum levels of VEGF and TNF-alpha and their association with C-reactive protein in patients with endometriosis. Arch Gynecol Obstet 2006; 273: 227-31.

[8] Revised American Society for Reproductive Medicine classification of endometriosis: 1996. Fertil Steril 1997; 67: 817-21.

[9] Barbieri RL, Niloff JM, Bast RC Jr, Scaetzl E, Kistner RW, Knapp RC. Elevated serum concentrations of CA-125 in patients with advanced endometriosis. Fertil Steril 1986; 45: 630-4.

[10] Somigliana E, Viganò P, Tirelli AS, Felicetta I, Torresani E, Vignali M, Di Blasio AM. Use of the concomitant serum dosage of CA 125, CA 19-9 and interleukin-6 to detect the presence of endometriosis. Results from a series of reproductive age women undergoing laparoscopic surgery for benign gynaecological conditions. Hum Reprod 2004; 19: 1871-6.

[11] Agic A, Djalali S, Wolfler MM, Halis G, Diedrich K, Hornung D. Combination of CCR1 mRNA, MCP1, and CA125 measurements in peripheral blood as a diagnostic test for endometriosis. Reprod Sci 2008; 15: 906-11.

[12] Hamilton JA, Iles RK, Gunn LK, Wilson CM, Lower AM, Grudzinskas JG. High concentrations of CA 125 in uterine flushings: influence of cause of infertility and menstrual cycle day. Gynecol Endocrinol 2002; 16: 19-25.

[13] Gagne D, Page M, Robitaille G, Hugo P, Gosselin D. Levels of vascular endothelial growth factor (VEGF) in serum of patients with endometriosis. Hum Reprod 2003; 18: 1674-80.

[14] McLaren J. Vascular endothelial growth factor and endometriotic angiogenesis. Hum Reprod Update 2000; 6: 45-55.

[15] Harada T, Iwabe T, Terakawa N. Role of cytokines in endometriosis. Fertil Steril 2001; 76: 1-10.

[16] Bulun SE, Yang S, Fang Z, Gurates B, Tamura M, Sebastian S. Estrogen production and metabolism in endometriosis. Ann N Y Acad Sci 2002; 955: 75-85; discussion 86-8, 396-406.

[17] Hyder SM, Nawaz Z, Chiappetta C, Stancel GM. Identification of functional estrogen response elements in the gene coding for the potent angiogenic factor vascular endothelial growth factor. Cancer Res 2000; 60: 3183-90.

[18] Martínez S, Garrido N, Coperias JL, Pardo F, Desco J, García-Velasco JA, Simón C, Pellicer A. Serum interleukin-6 levels are elevated in women with minimal-mild endometriosis. Hum Reprod 2007; 22: 836-42.

[19] Amaral VF, Ferriani RA, Sá MF, Nogueira AA, Rosa e Silva JC, Rosa e Silva AC, Moura MD. Positive correlation between serum and peritoneal fluid CA-125 levels in women with pelvic endometriosis. Sao Paulo Med J 2006; 124: 223-7.

[20] Pupo-Nogueira A, de Oliveira RM, Petta CA, Podgaec S, Dias JA Jr, Abrao MS. Vascular endothelial growth factor concentrations in the serum and peritoneal fluid of women with endometriosis. Int J Gynaecol Obstet 2007; 99: 33-7.

[21] Kianpour M, Nematbakhsh M, Ahmadi SM, Jafarzadeh M, Hajjarian M, Pezeshki Z, Safari T, Eshraghi-Jazi F. Serum and peritoneal fluid levels of vascular endothelial growth factor in women with endometriosis. Int J Fertil Steril 2013; 7: 96-9.

[22] Hormbrey E, Gillespie P, Turner K, Han C, Roberts A, McGrouther D, Harris AL. A critical review of vascular endothelial growth factor (VEGF) analysis in peripheral blood: is the current literature meaningful? Clin Exp Metastasis 2002; 19: 651-63.

[23] Leone R, Maggiore U, Ferrero S. Methodological concerns regarding levels of vascular endothelial growth factor (VEGF) in serum of women with endometriosis. Int J Fertil Steril 2015; 8: 485-6.