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Serum prokineticin-1 (endocrine gland vascular endothelial growth factor) as an alternative marker for assessing endometrium receptivity

I. ADENIN[1], D. LUTAN[1], T. TANDJUNG[1], H.K. SUHEIMI[2], M.F.G. SIREGAR[3]

SUMMARY: **Serum prokineticin-1 (endocrine gland vascular endothelial growth factor) as an alternative marker for assessing endometrium receptivity.**

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Backgrounds. *A total of ± 85-90% fertile couple will be able to conceive within one year. However, if there is no pregnancy within this period, even though the conjugal relationship is carried out regularly and without the use of contraception, a case of primary infertility occurs. Endometrium receptivity plays an important role in implantation process of conception product, and an optimal understanding about its function is crucial in infertility management.*

Purpose. *The purpose of this study was to determine the relationship between serum levels of PROK1 (EG-VEGF) to the expression of αvβ3 integrins in endometrial tissue to assess endometrial receptivity of fertile women with normal menstrual cycles.*

Method. *This study used a cross-sectional study design. This study was conducted in the outpatient unit of the Fertility and Reproductive Endocrinology Division (FER) of the Central General Hospital (RSUP) Dr. H. Adam Malik Medan, Department of Obstetrics and Gynecology, Faculty of Medicine, Universitas Sumatera Utara and Adenin Adenan Hospital Medan, starting in February 2017.*

Result. *42 samples were obtained in this study, with the majority of them came from age group of 20-35 years with 38 subjects (90.5%), normoweight BMI with 31 subjects (73.8%), age of menarche more at age < 13 years with 28 subjects (66.7%), and the duration of menstruation for 3-7 days with 40 subjects (95.2%). There were no significant differences in αvβ3 integrin expression based on age, BMI, age of menarche and duration of menstruation (p> 0.05). There were no significant differences in PROK-1 levels based on age, BMI, age of menarche and duration of menstruation (p> 0.05). There was a significant correlation between integrin expression of αvβ3 and PROK1 (EG-VEGF) with a value (p <0.001, r = 0.526).*

Conclusion. *There is no significant difference in the expression of PROK1 based on age, BMI, age of menarche and duration of menstruation but there is a significant correlation between PROK1 (EG-VEGF) serum level and integrin αvβ3 in fertile women.*

KEY WORDS: Serum PROK1 (EG-VEGF) - Endometrial receptivity - Fertile - Integrin αvβ3.

Introduction

Knowledge about reproductive process is the main modality for the treatment of infertility. This is because humans naturally want to conceive as an essential life cycle. A total of ± 85-90% fertile couple will be able to conceive within one year. However, if there is no pregnancy within that time period, even though the conjugal relationship is carried out regularly and without using contraception, primary infertility cases occurs. This occurs in ± 10-15% of partners, and this disorder is an important part of clinical practice (1, 2).

Today, many observations have been made for assessing endometrial factors, one of which is about endometrial receptivity to the implantation process of conception product. For this reason, observations and research on endometrial receptivity have been studied from various aspects over the past decade, including studies involving analysis of genetic-based proteomics, morphological markers of the endometrium, immunology factors, noninvasive examination using doppler ultrasonography in the endometrium, and examination using imaging technology (3, 4).

[1] Division of Reproductive, Endocrinology and Infertility, Obstetrics and Gynecology Department, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia
[2] Division of Reproductive, Endocrinology and Infertility, Obstetrics and Gynecology Department, Faculty of Medicine, Universitas Andalas, Padang, Indonesia
[3] Professor at Fertility and Reproduction Endocrinology Division, Department of Obstetrics and Gynecology, Adam Malik General Hospital, Faculty of Medicine, Universitas Sumatera Utara, Indonesia; President of Indonesian Menopause Association (Perkumpulan Menopause Indonesia)

Corresponding author: Ichwanul Adenin, e-mail: syahputra86.obgyn@gmail.com

However, recent studies proved that genetic-based proteomic analysis was considered as the most effective evaluation method that can explain the physiology and pathophysiology of organ systems in the body (5). One of them can be applied as a method for evaluating the receptivity of the endometrium as a target organ prepared for the implantation process of the conception product (6, 7).

The implantation process of conception product consists of three stages, namely: apposition, adhesion and invasion (6, 8). During the implantation process, there is a change in the ovarian steroid hormone levels (supportive to implantation window) around the 6^{th} to 10^{th} day after ovulation (9, 10). This process involves crosstalk synchronization between endometrial receptivity and functional blastocyst and a complex intercellular transduction signaling occurs due to the acquisition of ligand and *cell adhesion molecules* (CAM) (11, 12). Included in the CAM family are integrin cadherin selectin and immunoglobulin (13, 14).

αvβ3 integrin found in endometrial cell transmembrane will respond to *ligand binding*. Integrin aggregation formed discretely on cell surface membranes known as *focal adhesion sites* (FAS) will induce signaling of intercellular transduction because integrin-linked kinase (ILK) is formed (15, 16). Furthermore, with the effector modulation of the enzyme protein kinase, the specific glycoprotein hormone bound to the G *protein-coupled receptor* (GPCR) on the endometrial cell surface membrane will activates the *cyclic* AMP(cAMP) - protein kinase pathway, which causes a hormone secretion response and cause systemic effects including steroid hormone activity in the implantation window period that affects endometrial receptivity to the process of conception implantation (17, 18).

In addition, several studies have been conducted on proteins involved in the regulation of endocrine glands namely Prokineticin-1 (PROK1) which is involved in the process of angiogenesis, modulating the inflammatory response and regulation of hematopoiesis. Prokineticin-1 (PK1 or PROK1) is also called *Endocrine Gland-derived Vascular Endothelial Growth Factor* (EG-VEGF) (19, 20). Prokineticin-1 (PROK1 or EG-VEGF) is a member of the *family* prokineticin which also secretes the *mammalian Prokineticin-2 protein, venom protein A (VPRA)* from the *black mamba snake* and Bv8 protein from *frog Bombina variegate* (21, 22).

PROK1 (EG-VEGF) plays a role in the intracellular signal transduction pathway and sequential phosphorylation process by mediating the effect of PROK1 through PROK1 receptor (PROKR1) on the endometrium, where epithelial endometrial cells express *human* PROKR1 which also binds to G *protein-coupled receptor* (GPCR) (23, 24). One study also showed an increase in mobilization of the inositol triphosphate process stimulated by PROK1. Further observation shows the role of PROK1 and prokineticin-1 receptor (PROKR1) in human endometrium during the implantation process in early pregnancy (25).

Expression of PROK1 and PROKR1 is localized to the endometrial gland epithelium, luminal endometrium, endothelial and several compartments within the endometrial stroma, in addition it plays a role in the process of follicular development, at the time of initial implantation, in steroidogenic tissues, ovaries, testes, leydig cells, adrenal cortex, pancreas and prostate. It is also known that expression of PROK1 also plays a role in the regulation of steroid hormones (26, 27).

In the cycle that occurs in the ovary, PROK1 (EG-VEGF) is expressed by granulosa cells from the corpus luteum. In addition, the hormone progesterone and *human chorionic gonadotropin* (hCG) are associated with the expression of EG-VEGF in the endometrium (28, 29). This is what plays an important role in endometrial receptivity to prepare the blastocyst implantation process and maintain the optimal decidua in the early pregnancy (30).

Quantitative examination of PROK1 or immunoassay of Human EG-VEGF can be assessed from cell culture of endometrial tissue, also from blood specimens in the form of serum and plasma (31).

Levels of PROK1 (EG-VEGF) circulating in the blood are at high levels, especially in the initial luteal phase and remain until the mid luteal phase. If the level of PROK1 (EG-VEGF) is not optimal in this phase, it can inhibit the process of trophoblast invasion into the decidua, this occurs because the growth of the capillary plexus in endometrial tissue is inadequate, or not high (34, 35). This condition of non-receptive endometrium can be assessed from the expression of PROK1 in the endometrial tissue which is low in the phases *early* and *mid* luteal (32).

Based on the description above, a study of Serum Prokineticin 1 (Endocrine Gland Vascular Endothelial Growth Factor) was conducted as an alternative marker for assessing endometrial receptivity.

Methods

The study used *cross-sectional* design. This analysis was carried out in a univariate, bivariate and multivariate comparative analytical pairwise and correlative analytical variables. The study was conducted in the outpatient unit of the Fertility and Reproductive Endocrinology Division of the Central General Hospital (RSUP) Dr. H. Adam Malik Medan, Department of Obstetrics and Gynecology, Faculty of Medicine, Universitas Sumatera Utara and Adenin Adenan Hospital Medan, starting in February 2017 with a sample of 42 people.

The subjects that matched with the inclusion criteria were given an informed consent and signed the consent letter to follow the study. Subject data were recorded on the research questionnaire. All subjects performed ultrasound examination in TAS and TVS in mid cycle, to assess the uterus, endometrium, tuba and ovaries, and to exclude gynecologic organ disease that includes: uterine myoma, endometrial hyperplasia, and ovarian tumors or cysts. Transvaginal ultrasound (USG) examination (TVS) is performed using 3-D Honda, to assess the ovulation time, is when the diameter of the follicle is > 20 mm, the ovulation period is set 1-2 days after. This examination was done by the researchers themselves. Serum PROK-1 (EG-VEGF) examination was performed at the implantation window period on the 6th day until 10th after ovulation. Vein blood sampling from vena mediana cubiti (5cc) was performed by trained health officer (nurse/midwife), immunoassay examination was done in laboratory by examination method by ELISA method. Examination of αvβ3 integrin expression by performing an endometrial tissue biopsy at the implantation window period on days 6 to 10 after ovulation. Endometrial biopsy with biopsy technique according to operational standard procedure using AVM (Manual Vacuum Aspiration) with 5 mm cannula. Endometrial biopsy tissue is sent to Anatomy Pathology Laboratory of Faculty of Medicine Universitas Sumatera Utara Medan to examine expression of Integrin αvβ3 using immunohistochemistry method.

Results

42 patients who had met the inclusion and exclusion criteria were obtained. From Table 1, majority of subjects came from age group of 20-35 years with 38 people (90.5%). Most of the subjects were normoweight with 31 subjects (73.8%). Most subjects had menarche at age <13 years with 28 subjects (66.7%). Most of the subjects duration of menstruation were 3-7 days with 40 subjects (95.2%).

Differences in the levels of PROK-1 based on subject characteristics are shown in Table 2. There was no significant difference in PROK-1 levels based on age, BMI, menarche age and menstrual period (p> 0.05).

Table 3 shows the integrin expressions grouped into 2 categories, namely groups with negative and low intensity and groups with moderate to strong intensity. Based on age characteristics, it was seen that out of 4 subjects aged > 35 years, all had moderate to strong integrin expression. Whereas for subjects aged 20-35 years, from 38 subjects as many as 26 subjects (68.4%) were with moderate to strong expressions. By analysis using the Fischer's Exact test it was found that there was no relationship between age and integrin expression (p = 0.308).

Body mass index variables also did not have a significant relationship with integrin expression (p = 0.602). However, age of menarche has a significant relationship with integrin expression (p <0.001). Of the 28 subjects with <13 years of age, there were 26 subjects (92.9%) with moderate-strong integrin expression, while for subjects with menarche age ≥ 13

Table 1 - DEMOGRAPHIC CHARACTERISTICS OF RESEARCH SUBJECTS.

Demographic characteristics	**n = 42**
Age	
20 - 35 years	38 (90.5)
> 35 years	4 (9.5)
BMI	
Underweight	7 (16.7)
Normoweight	31 (73.8)
Overweight	4 (9.5)
Age of Menarche, n (%)	
<13 years	28 (66.7)
≥ 13 years	14 (33.3)
Duration of Menstruation, n (%)	
3 - 7 days	40 (95.2)
> 7 days	2 (4.8)

TABLE 2 - SERUM LEVELS OF PROK1 (EG-VEGF) BASED ON DEMOGRAPHIC CHARACTERISTICS.

Demographic characteristics	**n**	**PROK-1 (EG-VEGF), mean (SD)**	**P**
Age			
20 - 35 years	38	4.78 (2.15)	0.732[a]
> 35 years	4	5.08 (2.03)	
IMT			
Underweight	7	5.44 (2.04)	0.489[b]
Normoweight	31	4.63 (2.01)	
Overweight	4	5.13 (3.32)	
Age of Menarche, n (%)			
<13 years	28	4.45 (1.98)	0.421[a]
≥ 13 years	14	5.52 (2.28)	
Duration of Menstruation, n (%)			
3 - 7 days	40	4.67 (2.04)	0.742[a]
> 7 days	2	7.6 (2.4)	

[a]*Mann Whitney,* [b]*Kruskal Wallis*

TABLE 3 - EXPRESSION OF INTEGRIN αvβ3 BASED ON CHARACTERISTICS SUBJECTS.

		Expression of Integrin αvβ3		
Characteristics Subjects	**n**	**Negative-Low(0-1)**	**Medium-Strong(2-3)**	**P**
Age				
20-35 years			38 12 (31.6) 26 (68.4)	[a]>
35 years	4	0	4 (100)	
IMT				
Underweight	7	2 (28.6)	5 (71.4)	0.489[b]
Normoweight	31	8 (25.8)	23 (74.2)	
Overweight	4	2 (50)	2 (50)	
Age of Menarche, n (%)				
<13 years	28	2 (7.1)	26 (92.9)	0.421[a]
≥ 13 years	14	10 (71.4)	4 (28.6)	
Duration of Menstruation, n (%)				
3 - 7 days	40	10 (25)	30 (75)	0.742[a]
> 7 days	2	2 (100)	0	

[a]Fischer's Exact, [b]Chi Square

years, only 4 subjects (28.6%) with moderate-strong expressions. The menstrual length variable has no significant relationship with integrin expression (p = 0.077). Of the 40 subjects with 3-7 days of menstruation, 30 subjects (75%) had moderate-strong integrin expression. Whereas in subjects with longer menstruation > 7 days, none with moderate-strong expression.

Table 4 - RELATIONSHIP OF αvβ3 INTEGRIN EXPRESSION WITH SERUM LEVELS PROK1 (EG-VEGF)

	PROK-1 (EG-VEGF)	
	P	**R**
Integrin αvβ3	<0.001	0.526

From Table 4, using the Spearman correlation test, it was found that there was a significant correlation between integrin expression and PROK1 (EG - VEGF) with a value of p <0.001. The correlation value produced is 0.526 which means that integrin has a strong correlation and is positive with PROK-1. Positive values indicate that an increase in integrin expression will increase PROK-1 levels. The integrin and PROK-1 correlations can be seen in the Figure 1.

Discussion

Angiogenic growth factors, such as vascular endothelial cell growth (VEGF), induce endothelial cell proliferation, new gene expression, and directed cell migration as a key step in blood vessel formation new. This change was initiated by binding one of several forms of VEGF or related molecules to family members of the VEGF receptor (VEGFR). Of the 22 integrin heterodimers, at least six, αVb3, αVb5, α5b1, α2b1, αVb1, and α1b1, have been involved in angiogenesis (26, 27).

The relationship between the two systems is primarily correlative. The mechanism for direct communication between VEGF and integrins remains undefined and can largely reflect the convergence of their downstream intracellular signaling pathways. One common mechanism for regulating integrin function involves their activation. As a consequence of this activation, integrins increase their affinity or avidity for extracellular ligands (28).

In addition, there are a number of studies on VEGF with fertility. A study by Paul et al. involving 60 infertile women assessed the effect of serum VEGF levels on endometrial receptivity grade. Grading was assessed using histopathological examination at the middle of the luteal phase. This study also supports the results of this study, where they found a very significant difference (p = 0.001) between the mean serum VEGF levels and endometrial receptivity grade. Serum VEGF levels of 89 pg/ml can predict adequate endometrial receptivity, with a sensitivity of 89.5% and a specificity of 87.8% (29).

Zenneni et al. study of assessing uterine and subendometrial blood flow and EG-VEGF in women with unknown causes of infertility showed that EG-VEGF levels had a significant association with endometrial thickness and subendometrial pulses index.

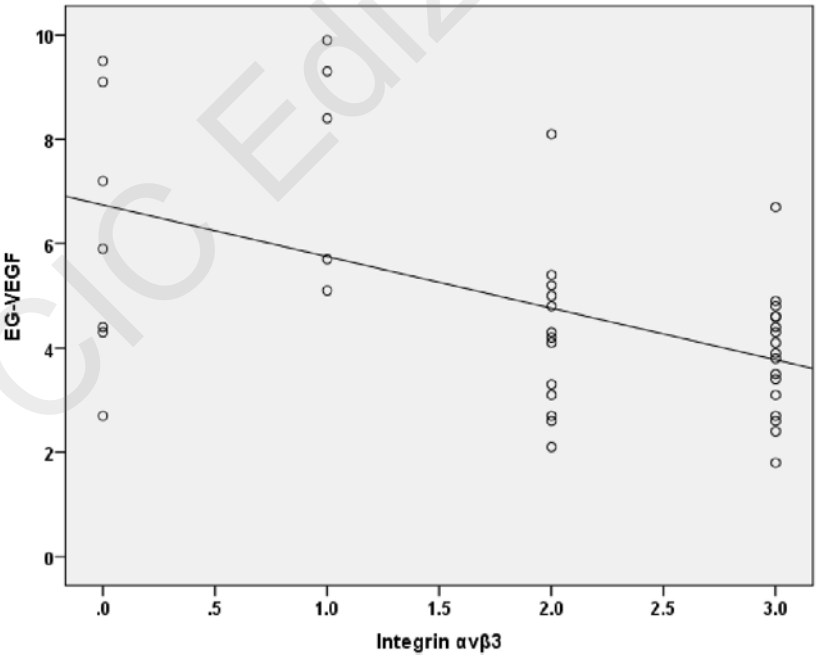

Figure 1 - Scatterplot Graph Correlation of Integrin Expression and PROK-1 (EG VEGF).

EG-VEGF is also significantly associated with infertility. The EG-VEGF ≤ 1 score had 64% sensitivity and 74% specificity in predicting infertility (30, 31).

Research conducted by Hannan et al. examined the dissolved mediator in uterine fluid, which states that VEGF is a key regulator of embryo implantation. This is because in this study endometrial epithelial cells given rh VEGFA showed > 5-fold increase in adhesion to fibronectin and 6-fold increase in adhesion to collagen. VEGFA levels were also significantly lower in infertile women compared to fertile women (31).

Research regarding the relationship between endometrial blood flow seen by Doppler examination and serum VEGF levels of infertile women conducted by Jain et al. showed that as the mean serum level of VEGF increases, the vascular penetration zone also increases, where this difference also has a significant value statistically. This is because VEGF is the main regulator of proliferation, angiogenesis, vasculogenesis, and capillary permeability in endothelium cells. Implantation is a progressive process in which the blastocyst will position, attach = and penetrate to the surface of the endometrium below. Angiogenesis is an important step for embryo implantation. Increased VEGF triggers the formation of capillary tissue so that good vascularization will support endometrial receptivity and improve the quality of embryo implantation (32).

In this study there was a significant correlation between integrin and PROK1 (EG-VEGF) expression ($p = 0.002$ with $r = -0.470$) which means that integrins have a moderate correlation and negative value with PROK-1.

From this result we can conclude that at endometrial receptivity is good in decreasing serum Prokineticine (PROK 1 or EG-VEGF) levels, this is assumed to be due to the large number of PROK 1 needed in the endometrium during the mid luteal period.

Conclusion

There were no differences in the expression of integrins αvβ3 based on age, BMI, age of menarche and duration of menstruation, but a significant correlation was found between integrin expression of αvβ3 and PROK1 (EG-VEGF).

Suggestions

There is a significant relationship between αvβ3 integrins and Serum PROK1 (EG-VEGF) levels showing an important role in endometrial receptivity for preparation of the implantation process. However, it is recommended to check quantitative PROK1 (EG-VEGF) receptor immunoassay from endometrial tissue.

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