Expression of VLA1, VLA2 and VLA3 Integrin Molecules in Uterine Endometrium of Infertile Women with Unexplained Aetiology in Ahwaz-Iran

Mehri Ghafourian Boroujerdnia[1*], Fathemeh Ghalambor Dezfuly[2], Nepton Emad Mosthophy[3], Rahim Chinipardaz[4]

[1]Department of Immunology, [2]Department of Obstetrics and Gynaeocology, [3]Department of Pathology, Medical College, Ahvaz Jondishapour University of Medical Sciences, [4]Department of Statistics, Shahid Chamran University, Ahwaz Iran

ABSTRACT

Background: Recent attention has focused on the expression of integrin molecules within the endometrium, and their relation to infertility. **Objective:** The present prospective study was undertaken to determine whether the endometrium of women with unexplained infertility differs in the expression of very late activation antigens (VLA) from the endometrium of normal fertile women. **Methods:** Thirty samples of endometrial biopsies from hysterectomies with non-endometrial pathology and 28 endometrial samples by uterine curetting from infertile women in secretary phase at implantation time were collected, stained with three monoclonal antibodies against β1 integrin subunits including VLA-1 to VLA-3 by immunohistochemical technique and then assessed semi-quantitatively by microscope. Chi-Square test was used to compare the expression of VLA antigens on epithelial cells, stromal cells, lymphocytes and vessels within endometrial tissues between two groups. **Results:** The results showed that most VLA integrins were present in fertile and infertile endometrium tissues. There were similarities and differences in the expression of VLA molecules in different compartments. VLA-2, VLA-3 expression on endometrial compartments showed an unaltered pattern of staining during the putative window of implantation in either fertile or infertile women with no significant differences (P-value> 0.5). VLA-1 expression on endometrial compartments changed in fertile and unexplained infertile women, the differences were related to the presence or lack of the molecules on epithelial and stromal cells respectively. **Conclusion:** Differences may explain causes of unexplained infertility, and suggests that certain integrins may participate in the cascade of molecular events leading to successful implantation and early placental development which requires more investigations.

Keywords: Endometrium, VLA integrins, Secretory phase, Infertility

*Corresponding author: Mehri Ghafourian, Department of Immunology, Faculty of medicine, Ahvaz Jondishapour University of Medical Sciences, Ahvaz, Iran. Tel: (+)98 611 3360245.e-mail:mehri_ghafourian@yahoo.com

INTRODUCTION

Infertility affects approximately 10-15% of couples in the reproductive age group (1). Evaluation of endometrial receptivity has been considered a basic goal in the evaluation of the infertile patients (2). It is now generally accepted that the endometrium is receptive to blastocyst implantation only during a short period in the luteal phase known as the implantation window (3). Several molecule markers have been shown to express on different endometrial compartments during this period (4, 5). Nowadays, interest in cellular adhesion molecules is increasing. Experimental works in animals and clinical studies have indicated that integrins may play a critical role in the process of implantation (6-8). Integrins are a widespread family of αβ heterodimeric membrane glycoproteins expressed on many non-haematopoietic cells as well as on leukocyte cell types. Integrin-mediated cell-cell and cell-matrix interactions regulate different type of cellular activities, including inflammatory response, angiogenesis, cell migration, proliferation, differentiation and gene expression. Integrins are classified into several groups according to their β subunit. The β1 integrin or VLA family has at least six distinct members (VLA1-6) which are expressed by a variety of cell types (9). β1 integrins have been shown to undergo specific changes within the endometrium during different phases of the menstrual cycle (10, 11). Due to the temporal pattern of their expression around the time of implantation and their absence in conditions related to infertility such as endometriosis, hydrosalpinges and unexplained infertility, these integrins might have an important role as a potential marker of uterine receptivity (12-14).

To confirm these observations and extend investigations of endometrial expression, we studied β1 integrin subunits including VLA1, VLA2 and VLA-3, using established monoclonal antibodies in a group of endometrial tissue specimens obtained from fertile and unexplained infertile patients.

MATERIALS AND METHODS

Tissues. Thirty samples of non-pregnant endometrium (Fertile cases) were obtained fresh from hysterectomies performed for non-endometrial pathology from the operating theatres. The inclusion criteria for fertile women including healthy and proven fertile; normal menstrual cycle and not used contraceptive drugs or intrauterine devices within the last 6 months.

Twenty eight endometrial samples were collected by uterine curetting from women with unexplained infertility from IVF Center. The inclusion criteria including infertility of a duration exceeding 1 year; normal quality sperm; normal ovulation; normal anatomical uterus and normal menstrual cycle.

All samples were obtained at the time of implantation window in secretory phase from Ahwaz Imam Khomaini Hospital.

Monoclonal Antibodies. Three monoclonal antibodies murine were used to stain immunohistochemically different β1 integrin markers in the all samples. All were suitable for use on frozen tissue sections. These were: monoclonal anti-human α1β1(VLA1) integrin which purchased from Biodesign Company, α2β1(VLA-2) and α3β1 (VLA-3) integrins supplied from Dako Ltd.

Immunohistochemical Staining. Acetone-fixed cryostat sections from endometrial biopsies were stained immunohistochemically with a range of monoclonal antibodies against β1 integrin subunits. Non-specific binding was inhibited by 10 minute incubation with normal rabbit serum. Tissue sections were incubated for 60 minutes with primary antibody, and stained with streptavidin-biotin-horse radish peroxidase system (Dako LSAB kit system). The sections were then stained by DAB enzymatic produced, counterstained with Mayer's haematoxylin and finally evaluated microscopically. Negative controls were incubated with irrelevant mouse monoclonal antibodies instead of primary antibodies. All incubations were carried out in a moist chamber at room temperature.

Scoring Method. The reactivity of antibodies directed against integrin subunits with different compartments of the endometrium (glandular epithelium, vessels, lymphocytes, macrophages, stromal cells) was scored semi- quantitatively according to the degree of positive staining: (-) when there was no reactivity greater than that observed in the negative control; (±) fewer than 5% of cells were positive; (+) 5-25% of cells were positive, (++) 25-50% of cells were positive; and (+++) more than 50% of cells were positive (10,11). Data were analyzed with the program Minitab version 10.0. Two groups were compared by chi-square analysis test.

RESULTS

The results showed VLA integrin subunits are present in fertile and infertile endometrium tissues (Table 1-3). There were similarities and differences in the expression of VLA molecules in different compartments. VLA-1 reactivity was detected in glands, vessel, lymphocytes and stromal cells. The reaction pattern for epithelial cells and stromal cells was significantly different between fertile and infertile endometrial tissues. In contrast to infertile cases, the reactivity for these compartments was positive for majority of fertile cases. The pattern of staining varied for lymphocytes and vessels between two groups but differences was not significant (P-value> 0.5).

Table1. Reactivity of VLA-1 with different compartments in secretory phase endometrium of fertile and infertile women

		-	±	+	++	+++
Glands	Fertile	0	0	0	10	20
	Infertile	0	6	14	3	5
Vessels	Fertile	0	0	13	17	0
	Infertile	0	0	6	21	0
Lymphocytes	Fertile	0	3	20	7	0
	Infertile	0	9	12	4	0
Macrophages	Fertile	30	0	0	0	0
	Infertile	28	0	0	0	0
Stromal Cells	Fertile	0	0	9	18	3
	Infertile	8	13	5	2	0

Key: (-) no reactivity; (±) <5%; (+) 5-25%; (++) 25-50; (+++) >50%

Table2. Reactivity of VLA-2 with different compartments in secretory phase endometrium of fertile and infertile women

		-	±	+	++	+++
Glands	Fertile	0	0	5	5	20
	Infertile	0	2	3	8	15
Vessels	Fertile	0	2	23	5	0
	Infertile	0	3	21	4	0
Lymphocytes	Fertile	30	0	0	0	0
	Infertile	28	0	0	0	0
Macrophages	Fertile	30	0	0	0	0
	Infertile	28	0	0	0	0
Stromal Cells	Fertile	30	0	0	0	0
	Infertile	28	0	0	0	0

Key: (-) no reactivity; (±) <5%; (+) 5-25%; (++) 25-50; (+++) >50%

Table3. Reactivity of VLA-3 with different compartments in secretory phase endometrium of fertile and infertile women

		-	±	+	++	+++
Glands	Fertile	0	0	0	4	26
	Infertile	0	0	3	15	20
Vessels	Fertile	0	0	9	19	2
	Infertile	0	2	10	16	0
Lymphocytes	Fertile	6	20	3	0	0
	Infertile	9	18	1	0	0
Macrophages	Fertile	30	0	0	0	0
	Infertile	28	0	0	0	0
Stromal Cells	Fertile	30	0	0	0	0
	Infertile	28	0	0	0	0

Key: (-) no reactivity; (±) <5%; (+) 5-25%; (++) 25-50; (+++) >50%

The reactivity for lymphocyte, macrophages and stromal cells with anti-VLA-2 antibody was negative. More than 50% of glandular epithelial cells in the majority of two group cases were positive with no significant differences. VLA-2 reactivity in 5-25% of vessels in most fertile and infertile cases was seen. There was not significant difference between two groups (P-value> 0.5).

Stromal cells and vessels reactivity for VLA-3 was more than 25% in majority of fertile and infertile endometrial tissues with no significant difference (P-value> 0.5). The expression of VLA-3 on lymphocytes was weak in all cases. Macrophages and stromal cells reactivity were negative.

DISCUSSION

The integrins we investigated in this study certainly do not represent the complete family of adhesion molecules, which play a role during implantation. The present immunohistochemical study showed that the VLA molecules are present in endometrium of fertile and infertile woman. Amongst the VLA molecules, only VLA-1 exhibited major changes in fertile and infertile endometrial tissues particularly in their expression by glandular epithelium and stromal cells. In most unexplained endometrium samples there was no or low reactivity for VLA-1 in glandular epithelial and stromal cells, which suggests this molecule may participate in the cascade of molecular events leading to successful implantation. Differences in β1 integrin

expression between fertile and infertile women have been shown by other researchers (6-8, 15). Differences in integrin expression between in- and out-of-phase endometrial biopsies were observed for αvβ3 integrin in glandular epithelial cells expression during the mid-luteal phase in women with impaired infertility (17). In opposite with above studies Thomas et al (18) in UK reported that there is no significant differences in integrin expression including α4β1, αvβ3 and α1β1 in endometrial biopsies, during implantation window, between patients attending for IVF and the control groups. Clearly, the IVF group consisted of patients with tubal disease, endometriosis, and unexplained infertility. In the UK, the waiting lists for IVF treatment on the National Health Serves (NHS) are invariably longer than 3 years, and a longer number of women would receive treatment during this time, perhaps improving the severity of the disease and therefore, in theory the integrin expression. On the other hand, stromal staining was not assessed in this study. A quantitative evaluation of a certain integrins by flow cytometry technique showed the defective expression of α1 integrin by endometrial stromal cells in unexplained infertile women in late secretory phase (7) that is in accordance with this study.

VLA-1 expressed weakly by glandular epithelium and stromal cells in proliferative phase and strongly in secretory phase, but negative in early pregnancy only in epithelial cells. The pattern of stromal and epithelial cell reactivity for VLA-1 did not change significantly between early and term pregnancy (10, 11, 15, 19). This pattern of reactivity for VLA-1 also reported for a4β1 and avβ3 during menstrual cycle (8, 11). Due to temporal pattern of their expression around the time of implantation and their absence in conditions related to infertility, these integrins might have an important role as a potential marker of uterine receptivity. Aberrant expression of these integrins has been found to be associated with endometriosis (20), hydrosalpinges (21) and unexplained infertility (14).

Several studies have demonstrated that β1 integrins can serve as receptors for laminin, fibronectin and collagen (22). From the secretory phase to early pregnancy the expression of laminin and collagen IV was increased in the pericellular matrix of stromal cells in normal endometrium (23). It is possible that the interaction between VLA-1-positive lymphocytes and laminin and collagen IV at these sites may lead to altered production of cytokines and expression of integrin molecules. Extracellular matrixes are able to present cytokines to cells. TGFβ, GM-CSF, IFNγ, TNFα, and IL-1β have all been demonstrated to alter the expression of integrin molecules (24). Cervical mucus TNFα concentration was found to be higher in idiopathically infertile women than in fertile controls (25). Increased T helper 1 cytokine responses in blood circulation have been reported in infertile women with multiple implantation failures after IVF (26). Cytokines like IL-1, CSF-1 and LIF involved in implantation processes (8, 27). PGE2 clearly enhances both β1 and β3 integrin subunit expression (28).

The pattern of reactivity for VLA-2 in present study suggests that it is unlikely this integrin has an important role in infertility. In contrast with this study, it has been reported VLA-2 reactivity in endometrial stromal cells (15). This finding may be attributed to the different monoclonal antibody used in the research. The reactivity for VLA-2 in the present study has been confirmed by several other studies (10, 19, 29, 30).

The majority of epithelial cells were positive for VLA-3 in both fertile and infertile endometrial tissues. Less than 5% of lymphocytes expressed VLA-3 in normal endometrium which was reduced in some infertile cases and may affects infertility. It has been reported a minority of lymphocytes expressed VLA-3 in secretory phase with

a slight increase in early pregnancy, possibly due to CD56-positive cells (31, 32). It has been reported that expression of α3β1, α4β1 and αvβ1 integrins in endometrium of women with recurrent miscarriages and women with unexplained infertility was similar in both groups (14).

During blastocyst implantation, interaction between integrins on the apical surface of the trophoblast and extracellular matrix (ECM) in the endometrium anchors the embryo to the uterine wall. It is not known how integrin signaling enhances blastocyst adhesion. Human trophoblast cells express laminan and fibronectin receptors, particularly α1β1, α5β1, α6β1, and α6β4. Adhesion of the trophoblast cells to extracellular matrix proteins blocked with antibody against integrins (33). It has been shown that the integrin, αIIβ3, plays a key role in trophoblast adhesion to fibronectin during mouse peri-implantation development (34). αvβ3, α4 and α5 integrin subunits, VN and FN are expressed in caprine endometrium and blastocyst and may play a role in the cascade of the implantation process (35). Recently difference in αv and beta 3 integrin subunits in the endometrium of women with tubal phimosis or hydrosalpinx (36) and unexplained infertility (37) have been emphasized and may serve as a marker of uterine receptivity in humans.

Considerable work is still required to elucidate the role of integrins in pathological situations in order to enhance the understanding of the mechanisms controlling implantation and early placental development.

ACKNOWLEDGMENTS

The authors are thankful to Ahwaz University of Medical Science for financial support of this research (Grant number 92).

REFERENCES

1 Randolph JF Jr. Unexplained infertility.Clin Obstet Gynecol. 2000;43:897-901.
2 Wentz AC, Kossoy LR, Parker RA. The impact of luteal phase inadequacy in an infertile population. Am J Obstet Gynecol. 1990;162:937-45.
3 Wilcox AJ, Baird DD, Weinberg CR. Time of implantation of the conceptus and loss of pregnancy. N Engl J Med. 1999;340:1796-9.
4 Giudice LC. Potential biochemical markers of uterine receptivity.Hum Reprod. 1999;14 :3-16.
5 Daftary GS, Taylor HS. Molecular markers of implantation: clinical implications.Curr Opin Obstet Gynecol. 2001;13:269-74.
6 Sueoka K, Shiokawa S, Miyazaki T, Kuji N, Tanaka M, Yoshimura Y. Integrins and reproductive physiology: expression and modulation in fertilization, embryogenesis, and implantation.Fertil Steril. 1997;67:799-811.
7 Illera MJ, Cullinan E, Gui Y, Yuan L, Beyler SA, Lessey BA. Blockade of the alpha(v)beta(3) integrin adversely affects implantation in the mouse.Biol Reprod. 2000;62:1285-90.
8 Lessey BA. Endometrial receptivity and the window of implantation.Baillieres Best Pract Res Clin Obstet Gynaecol. 2000;14:775-88.
9 Hynes RO. Integrins: versatility, modulation, and signaling in cell adhesion.Cell. 1992;69:11-25.
10 Tabibzadeh S. Patterns of expression of integrin molecules in human endometrium throughout the menstrual cycle.Hum Reprod. 1992;7:876-82.
11 Lessey BA, Castelbaum AJ, Buck CA, Lei Y, Yowell CW, Sun J. Further characterization of endometrial integrins during the menstrual cycle and in pregnancy.Fertil Steril.1994;62:497-506.
12 Szymanowski K, Skrzypczak J, Mikolajczyk M. Integrin pattern in human endometrium--new diagnostic tool in pelvic endometriosis?Ginekol Pol. 2003;74:257-61.
13 Yoshimura Y. Integrins: expression, modulation, and signaling in fertilization, embryogenesis and implantation.Keio J Med. 1997;46:16-24.
14 Skrzypczak J, Mikolajczyk M, Szymanowski K. Endometrial receptivity: expression of alpha3beta1, alpha4beta1 and alphaVbeta1 endometrial integrins in women with impaired fertility. Reprod Biol. 2001;1:85-94.
15 Klentzeris LD, Bulmer JN, Trejdosiewicz LK, Morrison L, Cooke ID. Beta-1 integrin cell adhesion molecules in the endometrium of fertile and infertile women.Hum Reprod. 1993;8:1223-30.

16 Tabibzadeh S. Implantation: from basics to the clinic.Ann N Y Acad Sci. 1997;828:131-6.
17 Creus M, Ordi J, Fabregues F, Casamitjana R, Ferrer B, Coll E et al. alphavbeta3 integrin expression and pinopod formation in normal and out-of-phase endometria of fertile and infertile women.Hum Reprod. 2002;17:2279-86.
18 Thomas K, Thomson A, Wood S, Kingsland C, Vince G, Lewis-Jones I. Endometrial integrin expression in women undergoing in vitro fertilization and the association with subsequent treatment outcome.Fertil Steril. 2003;80:502-7.
19 Bridges JE, Prentice A, Roche W, Englefield P, Thomas EJ. Expression of integrin adhesion molecules in endometrium and endometriosis.Br J Obstet Gynaecol. 1994;101:696-700.
20 Lessey BA, Castelbaum AJ, Sawin SW, Buck CA, Schinnar R, Bilker W, Strom BL. Aberrant integrin expression in the endometrium of women with endometriosis.J Clin Endocrinol Metab. 1994;79:643-9.
21 Meyer WR, Castelbaum AJ, Somkuti S, Sagoskin AW, Doyle M, Harris JE et al. Hydrosalpinges adversely affect markers of endometrial receptivity.Hum Reprod. 1997;12:1393-8.
22 Pigott R, Power C. The adhesion molecule, Facts book, Academic Press, London, England; 1993.
23 Reddy KV, Mangale SS.Integrin receptors: the dynamic modulators of endometrial function. Tissue Cell. 2003;35:260-73.
24 Nathan C, Sporn M. Cytokines in context.J Cell Biol. 1991;113:981-6.
25 Naz RK, Butler A, Witt BR, Barad D, Menge AC. Levels of interferon-gamma and tumor necrosis factor-alpha in sera and cervical mucus of fertile and infertile women: implication in infertility.J Reprod Immunol. 1995;29:105-17.
26 Kwak-Kim JY, Chung-Bang HS, Ng SC, Ntrivalas EI, Mangubat CP, Beaman KD et al. Increased T helper 1 cytokine responses by circulating T cells are present in women with recurrent pregnancy losses and in infertile women with multiple implantation failures after IVF.Hum Reprod. 2003;18:767-73.
27 Lindhard A, Bentin-Ley U, Ravn V, Islin H, Hviid T, Rex S et al. Biochemical evaluation of endometrial function at the time of implantation.Fertil Steril. 2002;78:221-33.
28 Pierro E, Minici F, Alesiani O, Monica LD, Anna MM, Muncuso S et al. In vitro regulation of beta1 and beta3 integrin subunits in endometrial epithelial cells from normal endometrium.Am J Reprod Immunol. 2003;49:373-6.
29 Ruck P, Marzusch K, Kaiserling E, Horny HP, Dietl J, Geiselhart A et al. Distribution of cell adhesion molecules in decidua of early human pregnancy. An immunohistochemical study.Lab Invest. 1994;71:94-101
30 Divers MJ, Bulmer JN, Miller D, Lilford RJ. Beta 1 integrins in third trimester human placentae: no differential expression in pathological pregnancy.Placenta. 1995;16:245-60.
31 Bulmer JN, Morrison L, Longfellow M, Ritson A, Pace D. Granulated lymphocytes in human endometrium: histochemical and immunohistochemical studies.Hum Reprod. 1991 Jul;6(6):791-8.
32 Vargas ML, Santos JL, Ruiz C, Montes MJ, Aleman P, Garcia-Tortosa C et al. Comparison of the proportions of leukocytes in early and term human decidua. Am J Reprod Immunol. 1993;29:135-40.
33 Burrows TD, King A, Smith SK, Loke YW. Human trophoblast adhesion to matrix proteins: inhibition and signal transduction.Hum Reprod. 1995;10:2489-500.
34 Rout UK, Wang J, Paria BC, Armant DR. Alpha5beta1, alphaVbeta3 and the platelet-associated integrin alphaIIbbeta3 coordinately regulate adhesion and migration of differentiating mouse trophoblast cells.Dev Biol. 2004;268:135-51.
35 Garcia P, Nieto A, Sanchez MA, Pizarro M, Flores JM. Expression of alphav, alpha4, alpha5 and beta3 integrin subunits, fibronectin and vitronectin in goat peri-implantation. Anim Reprod Sci. 2004;80:91-100.
36 Savaris RF, Pedrini JL, Flores R, Fabris G, Zettler CG. Expression of alpha 1 and beta 3 integrins subunits in the endometrium of patients with tubal phimosis or hydrosalpinx. Fertil Steril. 2006;85:188-92.
37 Ceydeli N, Kaleli S, Calay Z, Erel CT, Akbas F, Ertungealp E. Difference in alpha(v)beta3 integrin expression in endometrial stromal cell in subgroups of women with unexplained infertility. Eur J Obstet Gynecol Reprod Biol. 2006;126:206-11.