Study of αvß3 integrin gene expression in the endometrium of women with unexplained recurrent Spontaneous abortion

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ABSTRACT

Background: In 50% of cases, the pathogenesis of unexplained recurrent Spontaneous abortion (URSA) is still unclear. ` We assess the αvbeta3 integrin gene expression in fertile and RSA-afflicted women. **Methods:** Endometrial tissues (biopsies) (30 samples) divided into 15 RSA cases and controls (15) healthy through menstrual cycle in the mid-luteal period. The period of data collection was March 2022–December 2023. The αvß3 gene expression was examined and in compression to fertile women using (qRT-PCR). **Results:** The relative gene expression of αvß3 integrin was significantly decreased (p-value < 0.05) in RSA women in compression to control subjects. The reduction of αvß3 integrin might have an essential role in the pathogenesis of RSA. **Conclusion:** Comparing women with unexplained RSA to controls, the expression of integrin was considerably lower. Our results highlight the necessity of additional molecular examination of the endometrial tissue in afflicted women.

Keywords: URSA, αvß3 integrin, endometrial biopsy.

INTRODUCTION

Recurrent Spontaneous abortion (RSA), defined by some authors as 2 or more successive abortions, affects 1-3% of couples; an underlying reason is only identified in up to 50% of cases (1). They comprise immunogenic abnormalities, maternal features, genetic imbalances, and thrombophilic diseases, as well as embryonic factors (2). However, because the essential mechanisms are poorly understood, repeated pregnancy losses are classed as idiopathic when none of these risks are present. Because endometrial genes and proteins alter the endometrial microenvironment and may thus add to an aberrant fetal-maternal communication that results in gestation miscarriage, the efficient expression of these genes has been studied (3). Moreover, it has been discovered that appropriate implantation requires synchronization between embryonic development and endometrial decidualization. Numerous authors have investigated the contact between the embryo and the endometrium to clarify why pregnancies fail (4).

The appropriate endometrial gene expression through the menstrual cycle (mid-secretory period) has been known using global gene expression analysis (5).

Glycoproteins called integrin's are found on almost every cell surface and are involved in attachment and adhesion. The integrin's play in a series of proceedings leading to an effective establishment and gestation has drawn more and more attention. The expression of integrin changes during the endometrial cycle. Integrin expression is noticeably elevated at the moment of inserting (6). Precisely, the endometrium and embryo's progressive and spatial supply of integrin (αvβ3) in women corresponds with the attachment through implantation of the embryo (7). αvβ3 integrin is one

of these proteins. The combination integrin αvβ3 has been extensively studied in the human endometrium and functions as an adhesion promoter through contacts between cells with other cells. When implantation occurs, the αvβ3 integrin is produced in the glandular epithelium and translocated into the endometrial stroma, should pregnancy arise (8). A previous paper found no change in the αvβ3 integrin gene expression between RSA and fertile women (9). It would be significant to employ frozen endometrial biopsies sections to find a more perfect expression of the subunit of β3 integrin in instances with RSA. The integrin αvβ3 expression in endometrial frozen sections was reported by other scientists, who did not observe any differences between the groups (10). In contrast, some investigators observed that individuals with recurrent pregnancy loss had lower αvβ3 integrin levels through the window of implantation as compared to controls, either in microarray studies or freezing fragments (11). Technical disparities may be the cause of any discrepancy between researches.

Material and methods

In this case-control study, a total endometrial biopsy 30 samples (15 samples with recurrent miscarriage before 20 weeks of pregnancy and (15 samples) control group composed of women without abortion had at least one child. All samples were recruited from different infertility clinics, in Karbala governorate. Medical history, physical and chemical examination (including Cigarettes smoking and alcohol consumption, cytomegalovirus, thyroid gland issues, toxoplasmosis, metabolic disorders, autoimmune diseases, anti-phospholipid syndrome, polycystic ovaries, and anatomic abnormalities excluded by ultrasound analysis) were the criteria used to exclude patients from the study. The period of data collection was March 2022–December 2023. Each participant's age (25–40) and body mass index are similar.

Ethical Issues

The Research and Development Department of the Health Directorate in the province of Karbala approved the study. All participants have provided written informed permission following a thorough explanation of the study's goals.

Methods

Samples (Endometrial Tissue) were taken from all samples, through the menstrual cycle. Then, samples were kept directly in Trizol reagent at (− 20 °C), the optional ratio is 50-100 mg of tissue per 1 ml of Trizol. By using the RNA kit, total RNA was extracted. After that, cDNA synthesis according to cDNA kit protocol (Elabscience). Thus, the cDNA was then saved at (− 20 °C). qRT- PCR: quantitative real-time PCR was achieved by the BioFACT™ 2X real-time PCR Master Mix (Elabscience) on the cDNA samples by an Applied Bio-systems Step One ™ device. The amplification was achieved under the conditions: 14 minutes at 95 °C, 40 series of (95 °C) for 14 s, and 60 °C for 60 s, the negative control for all genes consisting of non-template water. The integrin αvβ3 gene primer was 5′-TAGAAGAGCCTGAGTGTCCTAAG-3′and 5′-TTCCAGATGAGCAGAGTAGCA-3′ (12). GAPDH, the housekeeping gene also named internal control gene used to normalize the relative gene expression. Also: to calculate relative gene expression by the equation $2^{\wedge} - \Delta\Delta Ct$.

Statistical analysis

According to (19) Data were analyzed using the software SPSS. The (M ± SD) of the mean independent sample T-test was used to express significance, with a $P < 0.05$.

Results

In this study, (15) RSA women and 15 fertile women were contributed. Table 1 shows the demographic and clinical features of all groups and αvβ3 gene relative expression. No significant change was detected for BMI and age between all samples ($p > 0.05$). The Results showed that the mean of the αvβ3 gene was expressed significantly decreased (0.69 ± 0.13) in the RSA group compared to the healthy women (1.15 ± 0.47).

Table 1 Characteristics of subjects.

Biomarkers / Groups	Age (years)	Number of Abortion	Number of Children	BMI (kg/m^2)	αvβ3 (relative expression)
RSA Patients (15)	31.93 ± 2.63	2.21 ± 0.43	NC	25.13 ± 0.72	0.69± 0.13*
Control (15)	32.33 ± 2.58	NA	2.13 ± 0.99	25.24 ± 1.08	1.15± 0.47
p. value <0.05 is significant.					

Discussion

We conducted this investigation in response to contradicting information regarding the αvβ3 gene expression in the RSA endometrium of women. Our results show that patients with URSA have significantly lower αvβ3integrin gene expression in comparison to control. The adhesion molecule function in feta-maternal communiqué through establishment is reinforced by the reduced integrin gene expression in the RSA endometrium (3). This result agrees with our study, by freezing sectors (13) or microarray investigations, other authors discovered that patients experiencing recurrent pregnancy loss had αvβ3 lesser levels through the window of grafting when compared to healthy women (14). Another recent paper found no change in β3 integrin expression between RSA and control (9), this paper disagrees with our study. The significance of this adhesion protein for implantation is demonstrated by the decreased expression of integrin in RSA. This decrease is consistent with other infertility-related diseases. For example, women with varying degrees of hydrosalpinges had low expression of integrin (15). Following; the removal of hydrosalpinges, αvb3 integrin levels rise, as well as gestation rates (3). The endometrial expressions of different Integrin were studied by a recent paper and correlated with different phases of the menstrual cycle (16). Later, other researchers showed that gene expression of the integrin αVβ3 increased, which could be important in promoting endometrial receptivity for embryo implantation (17). The embryo must be at the proper developmental stage (developmental window) and the endometrium must simultaneously reach the receptive stage (receptive window) for successful implantation (18). One of the most important stages of embryonic implantation is cellular adhesion and differentiation, which is mediated by heterodimer trans-membrane receptors called integrin's (16,19). Ultimately, molecular diagnosis using modern techniques, including DNA sequencing and real-time PCR measurement, is the best method for diagnosis (20).

Conclusion

Based on the current study's data, it is likely that αvβ3 expression is downregulated which may have an impact on the etiology of RSA. It still needs to do further research to determine the precise function of αvβ3 in the pathophysiology of RSA.

Limitation

To identify the role of relative gene expression of αvβ3 proteins more accurately, there are some limitations that we propose to address in future studies such as: (1) the number of samples was small; (2) Because of the difference in gene expression of αvβ3 proteins during the menstrual

cycle, we suggest studying gene expression in all phases of the menstrual cycle.

Conflict of interest: None

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