RESEARCH ARTICLE

Assessment of endometrial integrin beta 5 in women with unexplained recurrent pregnancy loss

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Abstract

Objective: To examine the relationship between endometrial integrin beta 5 level and risk of recurrent pregnancy loss.

Methods: The descriptive, prospective, observational, case-control study was conducted at the Kafrelsheikh University Hospital, Egypt, from January to May 2022, and comprised women aged up to 35 years with at least 1 live birth delivery beyond 20-week gestation with normal thyroid and prolactin levels. Age-matched normal fertile women were enrolled as controls. All the participants were subjected to detailed history and complete clinical examination. Endometrial integrin beta 5 was assessed using an antibody sandwich enzyme-linked immunosorbent assay. Data was analysed using SPSS 20.

Results: Of the 50 subjects, 25(50%) were cases with a mean age of 26.72±2.64 years, and 25(50%) were controls with a mean age of 25.36±2.16 years. The integrin beta 5 level was significantly lower among the cases than the controls ($p<0.05$). The best cut-off level of serum integrin beta 5 was ≤2.5765 with area under curve 0.886, sensitivity 88%, specificity 76%, positive predictive value 78.6%, negative predictive value 86.4%, and accuracy 82%.

Conclusion: There was an inverse correlation between endometrial integrin beta 5 and the risk of recurrent pregnancy loss.

Keywords: Pregnancy, Gynaecology, Obstetrics, Pathology, Abortion, Habitual, Endometrium, Enzyme, Immunosorbent assay, Integrins. **DOI: 10.47391/JPMA.EGY-S4-24**

Introduction

Recurrent pregnancy loss (RPL) is diagnosed by the presence of 2 or more consecutive pregnancy losses.[1] Causes are known in only 50% of such cases.[2] The known causes include embryonic factors, such as poor-quality embryos, chromosomal and genetic abnormalities.[3] Also, there are a lot of maternal factors, such as uterine malformations, antiphospholipid syndrome, hyperprolactinaemia, hypothyroidism and thrombophilia diseases.[4]

When no cause can be identified after full assessment, the RPL is classified as unexplained**[4].** There are a lot of genes and proteins in the endometrium that may affect its environment and thus affect the endometrial function, causing pregnancy loss.[4]

Global gene expression analysis[5] has found some endometrial genes and proteins during implantation window which is defined as the period following ovulation by 7-9 days.[6]

A lot of endometrial adhesion molecules have been shown to occur on the surface of endometrial cells. One of the endometrial proteins is integrin beta 5 (ITGB5), which acts as adhesion promoter via cell-to-cell interaction, and can combine with different alpha chains, forming a variety of integrin heterodimers.[7]

Some studies have examined unexplained RPL, and one of them studied relation between RPL and integrin alpha V beta 3 (αVβ3) in endometrial biopsy.[8]

The current study was planned to examine the relation between endometrial fluid ITGB5 and unexplained RPL.

Patients and Methods

The descriptive, prospective, observational, case-control study was conducted at the Kafrelsheikh University Hospital, Egypt, from January to May 2022. After approval from the institutional ethics review committee, the sample was raised from among those visiting the Obstetrics and Gynaecology Department. Those included were women aged up to 35 years having at least 1 live birth delivery beyond 20 weeks of gestation with normal thyroid function and prolactin level. Those aged >35 years, or had thyroid disease, antiphospholipid syndrome, thrombophilic abnormalities, chromosomal abnormalities, hyperprolactinemia and abnormal uterine anatomy were excluded.

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G**roup** A had case with 2 or more previous recurrent first trimester miscarriages, while group B had healthy fertile women who acted as controls. Written informed consent was obtained from all the participants, who were subsequently subjected to detailed history and full clinical examination.

For the cases, investigations were done to exclude the known RPL causes, like Thyroid Stimulating Hormone (TSH), Triiodothyronine (T3), Thyroxine (T4), lupus anticoagulant, anticardiolipins, anti-beta 2 glycoproteins, antithrombin 3, protein C, protein S, factor V Leiden gene mutation, karyotyping, serum prolactin, and transvaginal ultrasound.

Endometrial fluid was taken from both groups during the implantation[9] 7 days after ovulation, which was diagnosed clinically by transvaginal ultrasound**.**

The catheter of embryo transfer was attached to a 5ml syringe containing 3ml sterile saline which was infused into the endometrium slowly and aspirated, and the procedure was done four times till achieving turbulent flow and homogenous distribution of sample through the fluid. The samples were then frozen at -30°C. The endometrial samples were collected in tubes without any additive, and were stored at -30°C till ITGB5 level was analysed.

To assess ITGB5 concentrations in the samples, antibody sandwich enzyme-linked immunosorbent assay (ELISA) kit (Human ITGB5 ELISA kit, Catalog No. SG-12773) was used through an automated system (Best 2000 Biokit ELISA).

Data was analysed using SPSS 20. Data was expressed as mean standard deviation or frequencies and percentages, as appropriate. Student's t-test, chi square test and Fisher's exact test were used for various inter-group comparisons. Correlation coefficient was used to assess relationship between two variables. P<0.05 was considered statistically significant and p<0.01 was considered highly significant.

Results

Od the 50 subjects, 25(50%) were cases with a mean age of 26.72±2.64 years, and 25(50%) were controls with a mean age of 25.36±2.16 years. All the participants in both the groups had normal uterine anatomy and normal karyotyping (Table 1).

The ITGB5 was significantly lower among the cases than the controls (p<0.05), and the difference in term of parity and miscarriages was also significant between the groups (Table 2).

The best cut-off level of serum ITGB5 was ≤2.5765 with area under curve (AUC) 0.886, sensitivity 88%, specificity 76%, positive predictive value 78.6%, negative predictive value 86.4%, and accuracy 82% (Figure).

Multivariate regression analysis showed that increasing ITGB5 level was a protective factor (adjust odds ratio [AOR]: 0.045).

Table-1: Comparison between the groups regarding demographic and clinical characteristics.

Parameter	Group Case group (n=25) Mean ± SD	Control group(n=25) Mean ± SD	Test *t-value*	*p-value*
Age (year)	26.72 ± 2.64	25.36 ± 2.16	1.995	0.052
Weight (kg)	68.8 ± 4.56	67.32 ± 3.15	1.335	0.188
Ultrasound uterus				
Normal	25 (100%)	25 (100%)	0	>0.999
karyotyping:				
normal	25 (100%)	25 (100%)	0	>0.999
TSH (mIU/ml)	1.896 ± 0.331	1.91 ± 0.44	-0.123	0.903
T3 (ng/ml)	1.412 ± 0.207	1.476 ± 0.23	-1.035	0.306
T4 (ug/ml)	9.272 ± 0.953	9.844 ± 1.901	-1.975	0.054
Prolactin (ng/ml)	17.04 ± 2.81	15.28 ± 3.52	1.956	0.056
Lupus anticoagulant	0.91 ± 0.1	0.94 ± 0.08	-1.237	0.222
Anticardiolipin				
IgM (U/ml)	5.84 ± 1.15	6.08 ± 1.17	-0.731	0.469
Anticardiolipin IgG (ug/ml)	4.88 ± 1.02	5.4 ± 1.19	-1.674	0.101
Anti-β2 glycoprotein	2.78 ± 0.65	2.65 ± 0.52	0.768	0.446
Antithrombin 3 (%)	94.0 ± 4.05	96.04 ± 3.09	-2.002	0.051
Protein C (%)	85.2 ± 25.71	85.96 ± 3.55	-0.147	0.884
Protein S (%)	92.32 ± 5.07	91.4 ± 5.44	0.619	0.539
Factor V leiden mutation(negative)	25 (100%)	25 (100%)	0	>0.999

TSH: Thyroid stimulating hormone, IgM: Immunoglobulin M, t: Independent sample t test.

Table-2: Comparison between the groups regarding obstetric history and endometrial integrin beta 5.

Parameter	Group Case group (n=25) Median (IQR)	Control group (n=25) Median (IQR)	Test Z	*p-value*
Parity	0 (0 – 1)	2 (1 – 2.5)		
Range	0 – 2	1 – 3	-5.25	<0.001**
Nullipara	14 (56%)	0 (0%)		
Abortion	2 (2 – 4)	0 (0 – 0)	-6.312	<0.001**
Range	2 – 9	0 – 1		
Yes	25 (100%)	5 (20%)		
Integrin beta-5 (ng/ml):	2.11 (1.82 – 2.46)	2.91 (2.56 – 4.28)	-2.929	0.003*
Median (IQR)	1.54 – 3.36	1.92 – 5.52		
Range				

IQR interquartile range. **p≤0.001.

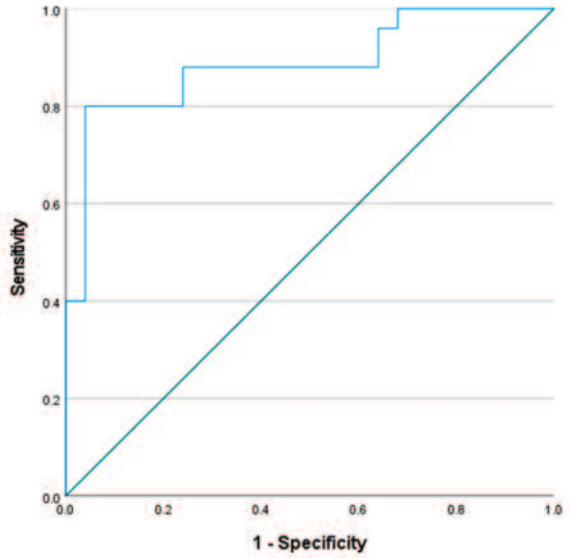

Figure: Figure: Receiver operating characteristic (ROC) curve showing performance of serum integrin beta 5 as a cause of recurrent pregnancy loss (RPL).

Discussion

RPL is a major health problem as 50% of the cases still remain unexplained. Integrins are type of proteins expressed in the endometrium. In the current study, the relation between the level of endometrial ITGB5 and RPL were examined as a less invasive way to measure integrins in endometrium to avoid endometrial damage, instead of the invasive endometrial biopsy.

The ITGB5 level was lower in RPL cases than healthy controls (Table 2).

Some studies examined the relation between RPL and endometrial integrins[8,9,10]. One of these studies[8] examined the presence of ITGB3 in endometrial biopsy in 21 RPL cases and 29 healthy controls, and found that the integrin level was reduced in the RPL group compared to the controls.

One study[11] examined the presence of mucin 1 (MUC1), leukaemia inhibitory factor (LIF) and ITGB3 in the biopsy of endometrial cavity of 14 women with history of recurrent implantation failure (RIF), 25 women with RPL history and 20 fertile controls. It found that MUC1 expression in the endometrium of RPL control groups was higher than the RIF group. There were no significant differences in LIF and ITGB3 levels among the three groups. In the current study, ITGB5 was examined in endometrial fluid, and the results were not in agreement with a previous study[9]. The reason behind this discrepancy may have been the way of biopsy collection or the sample size between the two studies.

Another study[12] examined the presence of beta-catenin (B-catenin), epithelial cadherin (E-cadherin) and kidney cadherin (K-cadherin) in endometrial biopsy of 40 women with primary infertility, 12 with RPL history and 24 fertile controls. It found that B-catenin, E-cadherin and K-cadherin expressions in endometrial cavity of fertile controls were significantly higher than the other groups, indicating that B-catenin, E-cadherin and K-cadherin can be used as markers of endometrial receptivity. The current study has suggested the use of endometrial ITGB5 as a marker of endometrial receptivity.

Most of studies that measured endometrial proteins used endometrial biopsy technique[8,11,12] to assess endometrial protein levels, but one study[10] used endometrial fluid technique to measure integrin αVβ3, vascular endothelial growth factor (VEGF), tumour necrosis factor-alpha (TNF-α) and LIF in 168 unexplained infertile women versus 169 normal fertile controls. The study[10] found that integrin αVβ3, VEGF, TNF-α and LIF levels in the endometrial fluid were significantly lower in the infertile group, and that endometrial integrin αVβ3 had the best predictive value of endometrial receptivity. The current study also used the endometrial fluid technique, but measured ITGB5 in RPL

cases versus normal fertile controls, and found that ITGB5 was higher in the controls, indicating that it can be used as a marker of endometrial receptivity.

The current study has its limitations as the sample size was not calculated, and it did not measure endometrial proteins other than ITGB5. As a case-control study, it could not calculate the absolute measure of association, could not differentiate between association and causation as the data was collected after the outcome, which also leads to recall and selection biases.

Conclusion

There was a strong correlation between endometrial ITGB5 and RPL risk. Endometrial ITGB5 can be used as a marker of endometrial receptivity.

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Conflict of Interest: None.

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