The Importance of CD56 and CD98 Levels in Patients with Recurrent Implantation Failure

CD56 ve CD98 Düzeylerinin Tekrarlayan Implantasyon Başarısızlığındaki Önemi

Implantation Failure

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Özet

Amaç: Yardımcı üreme tekniklerinde gözlenen büyük gelişmelerin aksine klinik gebelik elde etme oranları taze embriyo transferlerinde %31, oosit donasyonlarında ise %41 düzeylerinde kalmıştır. İmplantasyon penceresi denilen dönem ve implantasyonun kendisi in-vitro fertilizasyon uygulamasında önemli bir yere sahiptir. Bu çalışma ile tekrarlayan implantasyon başarısızlığı olan in-vitro fertilizasyon hastalarında CD56 ve CD98 in immunohistokimyasal olarak boyanmasındaki farklar incelenmek istenmiştir. Gereç ve Yöntem: Bu çalışma 2004 ile 2010 yılları arasında in-vitro fertilizasyon yöntemi uygulanan 6260 hasta verisinin bulunduğu bir veribankasından tarama ile seçilen 36 hastanın örneklerinin değerlendirilmesi ile yapılmıştır. Tekrarlayan implantasyon başarısızlığı en az 3 kez in-vitro fertilizasyon denemesi yapılması ve toplamda 8 embriyo verilmesini takiben b-HCG testinin pozitifleşmemesi olarak tanımlanmıştır. Diğer yanda kontrol grubunda ise ilk in-vitro fertilizasyon denemelerinde b-HCG testinin pozitifleştiği hasta grubu bulunmaktadır. Bulgular: Yapılan karşılaştırmada CD 56 ve CD 98 boyanma yüzdeleri, boyanma gücü ve boyanma skoları arasında gruplar arasında anlamlı farklar saptanmıştır. (p<.001). Tekrarlayan implantasyon başarısızlığı olan hastaların endometriyal örneklerinde CD 98 boyanması kontrol grubuna gore anlamlı olarak daha azdır. Tartışma: CD 56 ve CD 98 in immunohistokimyasal olarak boyanmaları in-vitro fertilizasyon tedavisi uygulanacak olan hastalarda tanısal testlerin parçaları olabilirler. Bu markırların gebelik oluşumu ve gebelik sürecine olan genel etkilerinin araştırılması açısından daha ileri araştırmalara gerek duyulmaktadır.

Anahtar Kelimeler

CD 56; CD 98; In-Vitro Fertilizasyon; İmplantasyon Başarısızlığı

Abstract

Aim: Despite major advances in assisted reproductive techniques, clinical pregnancy rates remain around 31% with fresh embryo transfer and around 41% with oocyte donations. We also know that the implantation process itself and the window period defined as the ''implantation phase'' are significantly important for successful in-vitro fertilization (IVF) cycles. With this study we have tried to determine any differences in immunohistochemical staining for CD56 and CD98 within the implantation phase endometrium of patients with recurrent implantation failure and of a control group that eventually had a successful IVF cycle. Material and Method: This study was retrospectively performed on a total of 36 patients selected out of a database of 6260 patients who received their IVF cycles from 2004 to 2010. Patients were defined as implantation failure if they did not have a positive result for b-HCG testing following at least 3 IVF cycles with a total of at least 8 embryo transfers. The control group was formed with patients who had success (positive b-HCG testing) on their first IVF treatment. Results: Comparison of means for CD 56 staining percentages, CD 98 staining percentages, CD 98 staining power, and CD 98 staining score showed significant difference between the control group and the study group (p<.001). The endometrium of patients without recurrent implantation failure is significantly more stainable by CD 98 than that of patients with recurrent implantation failure. Discussion: We suggest that CD 56 and CD 98 staining for endometrium tissue can be a part of diagnostic testing for patients who are candidates for IVF treatments. We need further studies to determine the correlation between the overall chance for pregnancy and these types of immunohistochemical staining for patients receiving IVF treatment.

Keywords

CD 56; CD 98; In-Vitro Fertilization; Implantation Failure

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Introduction

Implantation is one of the most important parts of the whole process that lies beneath the miraculous event of pregnancy. Implantation is a limiting factor for both natural (unassisted) female reproduction and in-vitro fertilization (IVF) cycles. Despite major advances in assisted reproductive techniques, clinical pregnancy rates remain around 31% with fresh embryo transfer and around 41% with oocyte donations [1].

CD 98 is a type II glycoprotein usually found in ovarian, testicular, and placental tissues. It also can be found in liver, renal, and splenic tissues, participating in the process of amino-acid and hormonal transport [2-3]. CD 98 expression, which is specific to the implantation phase for human reproduction, has a very important role. It was determined that rats with suppressed CD 98 expression with lent viruses showed a diminished blastocyst adhesion. Conversely, increasing CD 98 expression by 2-10 times showed an implantation rate up to 100% [4].

CD 56 positive cells, known as natural killer cells, have been studied extensively to understand their role in both implantation failure and recurrent miscarriages. The principal finding has been elevated numbers of CD 56 + cells in the endometrium of women with implantation failure and recurrent miscarriage compared to control groups [5-7].

As we know, many factors play a role in implantation failure. With this study we have tried to identify any differences of immunohistochemical staining for CD56 and CD98 within the implantation phase in the endometrium of patients with recurrent implantation failure and in the control group who eventually had a successful IVF cycle.

Material and Method

This study was performed retrospectively on a total of 36 patients selected out of a database of 6260 patients who had their IVF cycles between 2004 and 2010. 936 patients were categorized as implantation failure, which was defined as failure to have a positive result for b-HCG testing following at least 3 IVF cycles with a total of at least 8 embryo transfers. Note that the IVF treatments of the patients selected for this study occurred before the national law limiting the number of embryos that may be transferred under different circumstances went into effect.

Patient Selection

586 patients who did not have a hysteroscopy before their IVF cycle and 118 patients whose hysteroscopy revealed polyps, septum, or adhesions were excluded from the study. 41 patients with poor ovarian reserve, defined as the need for r-FSH more than 3000 units and/or patients with metaphase 2-oocyte count less than 6 in an IVF cycle, were also excluded. Additionally, 78 patients who were more than 37 years old were excluded due to advanced maternal age. Of the remaining 113 patients, 67 had a specimen taken from the endometrium but only 21 had 5-10 days post ovulatory characteristics defined by Noyes et al [8].

In selecting the control group, we retrospectively screened 3738 patients who were not successful in having spontaneous pregnancy but had pregnancy over 12 weeks on IVF cycle following an endometrial biopsy collected during the luteal phase. Of these, 2789 patients were excluded because they did not have a hysteroscopy and 403 patients were excluded because of pathological findings in the hysteroscopy. 237 patients were excluded because of advanced maternal age and 117 were excluded for poor ovarian reserve. 15 out of the 192 remaining patients had specimens appropriate for the implantation phase (post ovulatory 5-10 days).

Immunohistochemical Staining and Evaluation

5 µm samples were taken to Poly-L-Lysine covered microscopic slides from paraffin embedded original tissues. Following deparaffinization, ''Autostainer Link 48 (DAKO)'' and as antibody (7.0ml, Ready to use, Code IR 628, Clone 123C3, DAKO, USA) used for staining procedure for CD 56.

Image 1 shows sample images for CD 56 staining.

CD 98 staining was done with the biotin immuneperoxidase method using N1C2 (1/200 dilution, catalogue GTX 104108, Gene Text, Inc.) and incubated at +4°C overnight.

Image 2 shows sample images for CD 98 staining.

CD 56 antibody staining was scored from 0 to 4 according to the percentages of red-brown staining cell diffusiveness from 0%, 1%-25%, 26%-50%, 51%-75%, and 76%-100%, respectively.

CD 98 antibody staining was evaluated for its staining power and diffusiveness. Diffusiveness was scored as it was for CD 56 and the results were multiplied by a power score ranging from 1-3 as weak, moderate, or strong staining. The minimum score for CD 98 was 0 (no staining) and the maximum score was 12 (more than 75% and strong membranous staining). Stromal staining for CD 98 was recorded separately as positive or negative.

Independent samples T test, one-way ANOVA, and chi-square statistical methods were used as appropriate. IBM SPSS 17.0 was used for statistical analysis and a p value less than 0.05 was accepted as statistically significant.

Ethics and Institutional Review Board Approval

Baskent University IRB approved this study; the approval number is KA11/120.

Results

The study group had a mean age of 31.9 ± 2.7, whereas the control group had a mean age of 28.5 ± 3.5 (p=.002). Table 1 compares other IVF cycle parameters.

Comparison of means for CD 56 staining percentages, CD 98 staining percentages, CD 98 staining power, and CD 98 staining scores showed significant difference between groups (p<.001) (Table 2).

There were significant differences between subgroup results of CD 56 staining percentages, CD 98 staining power, and CD 98 staining percentages. No significant difference was observed in CD 98 stromal staining (Table 3). Using a scoring system that included both CD 98 staining percentage and CD 98 staining power (percentage value from 1-4 multiplied by power from 1-3), there was a significant difference between groups (p<0.05).

Table 1. Comparison of different IVF parameters between groups.

	Study Group	Control Group	P value
Total Transferred Embryo Count	11.95 ± 3.81	2.80 ± 0.56	<.001
D3 FSH (mIU/mL)	5.97 ± 1.53	4.91 ± 1.21	.043
Antral Follicle Count	6.61 ± 2.50	7.7 ± 3.20	.299
D5 E2 Level (pg/mL)	361.9 ± 222.1	420.4 ± 219.5	.453
HCG Day E2 Level (pg/mL)	1899.1 ± 1018.3	2114.3 ± 1007.1	.547
HCG Day Progesterone Level (ng/mL)	0.744 ± 0.53	0.671 ± 0.320	.635
HCG Day Endometrial Thickness	11.02 ± 2.10	11.54 ± 2.80	.531
Salvaged Oocyte Count	15.4 ± 6.0	15.4 ± 6.6	.914
Metaphase II Oocyte Count	12.6 ± 4.9	12.4 ± 5.9	.905
Fertilization Rate	66.3 ± 20.1	73.7± 21.6	.302
Treatment Length (Days)	9.19 ± 1.60	8.40 ± 1.24	.495

Table 2. Comparison of staining power and percentages of CD 98 and CD 56

	Study Group	Control Group	P Value
CD56 Staining Percentage	2.00 ± 0.77	1.20 ± 0.41	.001
CD98 Staining Percentage	2.33 ± 0.79	3.47 ± 0.64	<.001
CD98 Staining Power	1.57 ± 0.50	2.40 ± 0.50	<.001
CD98 Score	3.62 ± 1.53	8.20 ± 1.89	<.001

Table 3. Comparison of results within subgroups for CD 56 staining percentages, CD 98 staining power and percentages.

	Subgroups	Study group	Control group	P value
CD56 Staining Percentage	1 (1-25%)	33.3%	80%	.002*
	2 (26-50%)	42.9%	20%	
	3 (51-75%)	28.6%	0%	
CD98 Staining Power	1	42.9%	0%	<.001*
	2	57.1%	60%	
	3	0%	40%	
CD98 Staining Percentage	1 (1-25%)	9.5%	0%	<.001*
	2 (26-50%)	57.1%	6.7%	
	3 (51-75%)	23.8%	40%	
	4 (76-100%)	9.5%	53.3%	
CD98 Stromal Staining	Positive	9.5%	20%	.337
	Negative	90.5%	80%	

Discussion

Knowing the limitations of retrospective studies, we excluded possibly important factors for implantation such as poor ovarian reserve, patients with anatomical uterine defects and pathological findings, and advanced maternal age in order to improve the study quality. Also, the study and control groups had quite similar IVF cycle parameters, which contributed to comparability of groups.

There are authors who propose that a similar pathogenesis lies beneath recurrent implantation failure and recurrent pregnancy losses. That is the reason given by most of the leading articles, mostly done by Quenby et al., for recurrent implantation failure was done with CD 56 [7,9]. While Quenby et al. propose that implantation failure relates to oxidative stress on endometrial tissue originating from endometrial edema formation, which is found with increased number of uNK cells and endometrial blood vessels [9]. On the other hand, Matteo et al. found no difference in CD 56 + cells between the recurrent implantation failure group and the control group in a study performed with the flow cytometry technique which also includes CD 56 + cells from blood vessels [10]. Our study showed that the presence of CD 56 + cells in endometrial stromal tissue can be a destructive element for embryo implantation.

Although there is not sufficient published data to draw conclusions about human endometrial tissue, animal studies showed the importance of CD 98 expressing cells for implantation and reproduction. We found that a decreased number of CD 98 expressing cells, resulting in decreased amino-acid and hormonal transportation in endometrial tissue, can be one of the factors for implantation failure.

We suggest that CD 56 and CD 98 staining for endometrium tissue can be a part of diagnostic testing for patients who are candidates for IVF treatments. We need further studies to determine the correlation between the overall chance for pregnancy and these types of immunohistochemical staining for patients receiving IVF treatment.

Competing interests

The authors declare that they have no competing interests.

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