Sirtuin 1 Levels in Recurrent Implantation Failure

Níveis de sirtuína 1 em falhas recorrentes de implantação

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Abstract

Sirtuin 1 has an important role in cellular processes, including apoptosis and cellular stress. The purpose of this study was to assess serum sirtuin 1 levels in women with recurrent implantation failure (RIF). In this cross-sectional study, we included 28 women with RIF, 29 healthy women who had conceived by in vitro fertilization (IVF), and 30 women with a 1-cycle failure of IVF as controls. Human serum nicotinamide adenine dinucleotide (NAD)-dependent deacetylase sirtuin-1 (SIRT1/SIRT2L1) levels were detected using a commercial colorimetric kit. Recurrent implantation failure patients have higher sirtuin 1 levels than non-pregnant women and healthy pregnant women, but this difference did not reach statistical significance due to the low number of patients in our study. These higher sirtuin 1 levels may result from the inflammation imbalance of RIF patients. The only statistically significant correlation found was between age and sirtuin ($r = 0.277$, $p = 0.009$).

Keywords
► recurrent implantation failure
► sirtuin
► pregnancy
► in vitro fertilization

Resumo

A sirtuína 1 tem importante função nos processos celulares, incluindo a apoptose e o estresse celular. O objetivo deste estudo é o de avaliar níveis de sirtuína 1 em mulheres com falhas recorrentes de implantação (FRI). Neste estudo cruzado, incluímos 28 mulheres com FRI, 29 mulheres saudáveis que deram à luz por fertilização in vitro (FIV) bem-sucedida, e 30 mulheres com 1 ciclo de FIV malsucedido como controle. Os níveis de sirtuína 1 em soro humano de desacetilase dependente de dinucleotídeo de nicotinamida adenina (DNA) (SIRT1/SIRT2L1) foram detectados usando um kit colorimétrico comercial. Pacientes com FRI tiveram níveis de sirtuína 1 superiores às pacientes grávidas e aos controles, mas esta diferença não atingiu significância estatística devido ao baixo número de pacientes envolvidos. Estes níveis mais altos de sirtuína 1 podem ser resultado da inflamação desigual em pacientes com FRI. A única correlação estatisticamente significante encontrada foi entre idade e sirtuína ($r = 0,277$, $p = 0,009$).

Palavras-chave
► falhas recorrentes de inseminação
► sirtuína
► gravidez
► fertilização in vitro

Introduction

Successful implantation requires reciprocal interaction between the blastocyst and the endometrium. The maternal immune system has a role in this interaction, as well as the mediators secreted from the endometrium. The receptivity of the endometrium is crucial for processes of implantation and placentation. The evidence of implantation may be observed with an ultrasound examination. Recurrent implantation failure (RIF) is an important problem in in vitro fertilization (IVF)/intracytoplasmic sperm injection (ICSI) cycles.[1,2] Along with different definitions, RIF is defined as implantation that has repeatedly failed to achieve a clinical pregnancy after a cumulative transfer of more than 3 good-quality embryos in at least 3 fresh or frozen unsuccessful cycles in a woman under the age of 40 years.[3] Decrease in endometrial receptivity, uterine factors, embryonic defects, immunological factors, factors associated with thrombophilia, and other combined factors can be defined in RIF etiology.[2,4] The balance state of pro- and anti-inflammatory cytokines is crucial for implantation and to maintain the pregnancy.[5] Pro-inflammatory cytokines are increased in RIF.[3]

Sirtuin 1, also known as nicotinamide adenine dinucleotide (NAD)-dependent class III protein deacetylase,[6] plays an important role in cellular processes, including gene expression, cell cycle regulation, cell metabolism, oxidative stress response, apoptosis, cellular stress, DNA repair and aging.[7–9] Sirtuin 1 also has effects in chronic inflammation and immune response.[10,11] The aim of this study is to define the effect of sirtuin 1 levels in RIF patients.

Methods

This cross-sectional study sample consisted of 87 women. The study was conducted according to the Declaration of Helsinki.[12] The institutional review board (# 26/6/2014) approved the study. The RIF group included 28 infertile women. Recurrent implantation failure was defined as failure to achieve a clinical pregnancy after a cumulative transfer of at least 3 good-quality embryos in at least 3 fresh or frozen unsuccessful cycles in a woman under the age of 40 years. The two control groups included 29 healthy women who had conceived by in vitro fertilization (IVF), and 30 women with a 1-cycle failure of IVF. Age, body mass index (BMI), antral follicle count (AFC), estradiol, follicle-stimulating hormone (FSH), luteinizing hormone (LH), prolactin and thyroidstimulating hormone (TSH) levels were evaluated. Blood samples were obtained in the morning from the 1st to 3rd days of the menstrual cycle, following at least 8 hours of fasting. Centrifugation of the samples was performed at 3,000 rpm for 10 minutes, and the supernatant serum was separated and stored at $-80°C$ until the assays were performed. The BMI was calculated in all of the patients and controls as kg/m^2. Commercial colorimetric enzyme-linked immunosorbent assay (ELISA) kits (Hangzhou Eastbiopharm, Hangzhou, China) were used for the measurement of human serum NAD-dependent deacetylase sirtuin-1 (SIRT1/SIRT2L1) using appropriate wavelengths and a microplate reader (EL X 800 TM, BioTek Instruments, Winooski, Vermont, US),

according to the manufacturer's instructions. The concentrations were calculated over the standard curves. The intra-assay precision was CV (Coefficients of Variability) % < 8%, and the inter-assay precision was CV % < 10%. The assay range was 0.2–60 ng/mL, and the sensitivity of the assay was 0.09 ng/mL. The kit uses a double-antibody sandwich ELISA. The results were expressed as ng/mL of protein. Complete blood count, and fasting plasma glucose (FPG), FSH, LH, estradiol, prolactin and TSH levels were evaluated in all participants.

We tested the normality of the distribution of the variables using the Kolmogorov–Smirnov and Shapiro-Wilk tests. When the normality tests revealed normal distribution, one-way analysis of variance (ANOVA) was used followed by Bonferroni to compare the differences between two groups. When the test failed, comparisons of variables among the groups were performed using the Kruskal-Wallis non-parametric analysis of the variance, followed by Mann-Whitney U tests for pairwise comparisons. Demographic variables and test results were presented as mean with standard deviation (SD), or median with range for continuous variables. Pearson correlation coefficients were calculated for continuous variables with normal distribution, and Spearman rank correlation coefficients were calculated for non-normally distributed continuous variables. A two-tailed p-value < 0.05 was considered statistically significant. The Statistical Package for the Social Sciences (SPSS, IBM Corp., Armonk, NY, US), version 23.0, was used for the data analyses.

Results

There were no significant differences in terms of age, BMI, AFC, estradiol, FSH, LH, TSH and prolactin levels among the three groups (►**Table 1**). There were no significant differences in terms of hematological inflammatory parameters, white blood cells (WBCs), neutrophils, lymphocytes, mean platelet volume (MPV), platelet distribution width (PDW), and systemic inflammatory response (SIR) markers, such as the neutrophil/lymphocyte ratio (NLR) and the platelet/lymphocyte ratio (PLR) among the three groups (►**Table 1**). Recurrent implantation failure patients have higher sirtuin 1 levels than non-pregnant women and healthy pregnant women, but this difference did not reach statistical significance in the present study (►**Table1**). We found no correlation between any of the variables and sirtuin. The only statistically significant correlation found was between age and sirtuin (r = 0.277, p = 0.009).

Discussion

This is the first study investigating the association between sirtuin 1 and RIF. We found that the study groups did not differ in terms of the level of serum sirtuin 1. The process of implantation depends on different factors, such as the quality of the embryo, optimal culture conditions, the receptivity of the endometrium, and the maternal immune system.[13] According to data from studies performed with RIF patients, proinflammatory cytokines are increased, while anti-inflammatory cytokines are decreased,[3] and abnormal cytokine

Table 1 Characteristics of the three groups

	Non-pregnant (n = 30)	Pregnant (n = 29)	RIF (n = 28)	p
Age (years)	30.3 ± 5.1	31.1 ± 5.0	30.9 ± 4.9	0.811
BMI (kg/m^2)	24.5 ± 4.6	25.8 ± 4.2	25.5 ± 4.5	0.341
Estradiol (pg/mL)	42.5 ± 16.4	42.4 ± 13.1	36.2 ± 13.2	0.264
FSH (mIU/mL)	6.9 ± 2.3	8.5 ± 4.1	7.7 ± 4.7	0.348
LH (mIU/mL)	4.4 (1.5–11.7)	4.7 (0.9–28.4)	3.9 (1.8–19.4)	0.854
Prolactin (ng/mL)	14 (1.2–29.7)	11.9 (0.6–53.8)	12.7 (5.5–32.2)	0.508
TSH (mIU/mL)	2 (0.3–9.4)	1.6 (0.4–6.1)	1.9 (0.4–5)	0.154
AFC (n)	11.5 (4–22)	14 (2–22)	12 (7–22)	0.512
WBCs (x 10^3/µL)	7.4 ± 1.7	7.1 ± 1.6	7.2 ± 1.3	0.789
Neutrophils (x 10^3/µL)	4.7 ± 1.3	4.4 ± 1.4	4.4 ± 1.1	0.555
Lymphocytes (x 10^3/µL)	2.2 ± 0.6	2.1 ± 0.6	2 ± 0.6	0.629
MPV (fl)	16.1 (2.9–10.7)	16.1 (15.3–17.1)	15.9 (11.2–16.5)	0.368
PDW	9.9 (7.9–13.1)	9.5 (7.2–13.1)	9.8 (7.7–12.3)	0.121
NLR	2 (1.1–4.7)	2.1 (0.7–4.3)	2 (1.3–7.2)	0.993
PLR	130 (68.4–301.5)	137.6 (81.9–304.9)	142.1 (84.9–1160)	0.420
Sirtuin (ng/mL)	6.3 (2.2–37.1)	6.5 (2.8–26.3)	7.4 (2.7–36.0)	0.318

Abbreviations: AFC, antral follicle count; BMI, body mass index; FSH, follicle-stimulating hormone; LH, luteinizing hormone; MPV, mean platelet volume; NLR, neutrophil/lymphocyte ratio; PDW, platelet distribution width; PLR, platelet/lymphocyte ratio; RIF, recurrent implantation failure; TSH, thyroid-stimulating hormone (TSH); WBCs, white blood cells.

production causes RIF.[14,15] It is not the only factor in fetal and maternal unit hemostasis. These complex interactions change as the pregnancy progresses. The interactions between the fetal and maternal unit changes with the progression of pregnancy.[16] Moreover, apoptosis plays an important role in the progression of pregnancy.[17]

Sirtuin 1 reduces the apoptosis induced by DNA damage and oxidative stress. Shirane et al[18] revealed that the activation and increased expression of SIRT1 play an important role in uterine receptivity. Additionally, sirtuin 1 inhibits inflammatory pathways through macrophages, and is also effective in metabolic syndrome.[10] In an another study, mitochondrial dysfunction, oxidative stress and inflammation states were found associated with a decrease in sirtuin 1 levels in joint tissue.[19] Conti et al[20] concluded that inflammation and oxidative stress in chronic obstructive pulmonary disease is related to an increase in sirtuin 1 levels.

According to a study by Van Gool et al[21] in animals, sirtuin inhibits nicotinamide phosphoribosyl transferase and, after that, it reduces NAD levels, resulting in a decrease in the levels of tumor necrosis factor (TNF), interferon gamma (IFNγ), and in the amount of lymphocytes in the peripheral blood. Kong et al[22] also indicated that the activation of sirtuin 1 had a potential therapeutic function in autoimmune inflammatory diseases. On the other hand, Lugrin et al[23] expressed that the inhibition of sirtuin by pharmacological agents results in treatment of the acute and chronic inflammatory responses.

As a conclusion of this study, RIF patients have higher sirtuin 1 levels than non-pregnant women and healthy pregnant women, but this difference did not reach statistical significance due to the low number of patients in our study. This higher sirtuin 1 levels may result from the inflammation imbalance of RIF patients. Therefore, further studies with large samples are needed.

Conflicts of Interest
Authors declare no conflicts of interest.

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