European Review for Medical and Pharmacological Sciences 2016; 20: 5037-5040

Disturbed endometrial NF-κB expression in women with recurrent implantation failure

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Abstract. – OBJECTIVE: This study was planned to investigate whether expression levels of endometrial NF-κB1 and NFκB p65 changes in women with recurrent implantation failure (RIF).

PATIENTS AND METHODS: The study group consists of 30 RIF patients having at least three previous failed IVF cycles. The control group comprises of 30 patients having one or no previous failed attempt. Endometrial samples were obtained from all participants during hysteroscopy at the late follicular phase. Samples underwent ELISA analysis and immunohistochemical staining. The semi-quantitative H-Score method was used for analyzing the intensity of endometrial NF-κB p65 expression.

RESULTS: The concentrations of endometrial NF-κB1 were found to be significantly increased when compared to control subjects. Likewise, significantly increased NF-κB p65 immunoreactivity was detected in the cytoplasm of luminal and glandular epithelial cells. The H-Score of NF-κB p65 in RIF women was found to be significantly increased when compared to control group.

CONCLUSIONS: Increased levels of NF-κB1 and NF-κB p65 in the endometrium of RIF women can disturb physiological inflammation which is known to be positive modulator of endometrial receptivity.

Key Words:
NF-κB1, NF-κB p65, RIF, Inflammation.

Introduction

Blastocyst implantation is a coordinated process regulated by several numbers of inflammatory cytokines[1,2]. Accordingly, it is well-known fact that physiological amount of endometrial inflammation is necessary for successful embryo implantation[2]. In addition to critical role of sex steroids and receptivity genes on implantation, local endometrial inflammation is also required for endometrium-embryo interaction[2]. We can give any examples that showing the critical effects of inflammation on receptivity. By regulating synthesis and secretion of inflammatory molecules endometrial injury improves the expression of receptivity genes and cytokines[1]. Further, pathological endometrial inflammation arising from the existence of hydrosalphinges was found to be detrimental for embryo implantation[2]. Moreover, intra-uterine device leads to the accumulation of endometrial inflammatory cells and molecules that prevent implantation.

The existence of endometrial receptivity defect has been reported in women with recurrent implantation failure (RIF). Studies showed[3,4] that expression levels of endometrial receptivity genes and prostaglandins altered in women having RIF. Endometrium of fertile subjects is histologically in phase and normal in appearance. On the other hand, morphological evaluation of endometrium of RIF women might be normal in appearance but, in fact, they may show abnormality during histological or molecular analysis[2,5]. As supportive, the histological phase of the endometrium of RIF women is not consistent with the cyclical phase of subjects. Concordantly, underdeveloped endometrium has been reported in this subjects[5]. In line with this, Ruiz-Alonso et al[6] observed that the window of implantation displaced in RIF women.

Defective expression of NF-κB has been reported in some women suffering from infertility[7-9]. Accordingly a recent study conducted by Luo et al[9] showed that NF-κB gene polymorphism was noted to be associated with RIF. NF-κB is a transcription factor consisting of homodimers or heterodimers[10]. Both p50/p105 (NF-κB1) and p65 (Rel A) are two critical dimers of NF-κB. They are located in the cell cytoplasm. Both dimers are bounded by inhibitory protein IκBα. Extracellular signals activate NF-κB and phosphorylate IκBα. This reaction is responsible for releasing of NF-κB[11,12]. Both dimers bind to DNA, translocate into the cell nucleus and activate several molecules[13,14]. Although failed endome-

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trial receptivity has been reported in some patients with RIF the underlying mechanisms of disturbed implantation remain elusive. RIF not only leads to defective expression of receptivity genes but also may disturb expression of inflammatory molecules in the endometrium. When reviewing the literature, there is no study investigating endometrial NF-κB expression in women with RIF. This clinical and case-control study was planned to detect whether NF-κB1 and NFκB p65 concentration change in the endometrium of RIF women.

Patients and Methods

A total of 60 infertile participants were recruited for the present study. The study group consisted of 30 women diagnosed with RIF. Women had at least 3 failed cycles with transfers of at least two high-quality fresh or frozen-thawed embryos were defined as RIF. The control group consisted of 30 infertile women with the same age but who had only one or no previous failed attempt. Primary inclusion criteria for RIF and control subjects were the presence of normal ovarian reserve. For that reason, the total number of retrieved oocyte in previous cycles was taken into account. All RIF and control patients underwent transvaginal ultrasound examination, karyotypes analysis, and thrombophilia evaluation. Participants with the uni- or bilateral hydrosalpinges, submucous or intramural leiomyoma distorting endometrial cavity, endometrial polyp and hyperplasia, endometritis, uterine synechiae, severe endometriosis or ovarian endometrioma, hereditary or acquired thrombophilias were excluded. Subjects with male factor infertility were also excluded. In order to investigate possible endometrial etiology of previous failed IVF attempt and make a local endometrial injury both groups of participants underwent hysteroscopy. Endometrial samples were obtained from participants during hysteroscopy at the late follicular phase. The endometrial tissues were washed with a sterile saline solution to remove blood and transferred into RNA stabilization buffer and stored in -80°C for future analysis. The study was performed according to the guidelines of the Helsinki Declaration on human experimentation and informed consent was obtained.

Measurement of Endometrial NF-κB1 by ELISA

In both groups of participants, endometrial NF-κB1 levels were measured by enzyme-linked immunosorbent assay by using NF-κB1 ELISA kit. This kit has ability to detect NF-κB1 levels in the endometrium. Detailed information about the preparation of homogenized endometrial samples can be found elsewhere[15]. Kit has 0.31 to 20 ng/mL detection range. The intra and inter assay coefficients of variation were found to be <10% and <12%, respectively. The results of NF-κB1 were presented as ng/mg tissue.

Immunohistochemical Staining of Endometrial Samples

Paraffin slides containing endometrial tissues were cut four to five micrometer and incubated in hydrogen peroxide. The immunoreaction was realized about one hour with ready to use NF-κB p65 Ab-1 antibody. Following washing in PBS, the sections were incubated with horseradish peroxidase kit and stained with amino ethyl carbazole chromogen and hematoxylin. Human placenta obtained as the positive control. The detailed information regarding staining method can be found elsewhere[15].

Statistical Analysis

The normality of scattering of data, was performed by using Kolmogorov-Smirnov test, and all variables were found to abnormally distribute. The continuous variables were analyzed by Kruskal Wallis and Mann-Whitney U tests. The categorical data were analyzed by the Pearson X^2-test. For all comparisons $p < 0.05$ was accepted statistically significant. The results are expressed as the mean and standard deviation (SD). To calculate expression levels of endometrial NF-κB p65 H-Score method was used[15]. This is a semi-quantitative method consisting of the percentages of positively stained endometrial cells multiplied by a weighted intensity of staining: H-Score=Σ Pi (i+1), where Pi is the percentage of stained endometrial cells in each intensity category (0-100%), and i is the intensity indicating weak (i = 1), moderate (i = 2) or strong staining (i = 3)[15-17].

Results

The demographic characteristics, NF-κB1 concentrations, and H-Score of NF-κB p65 in each group of the participant, were presented in the Table I. Both groups of patients had a similar age. The concentrations of endometrial NF-κB1 were found to be significantly increased when compared to the control subjects. Immunohistochemical analysis of endometrial samples revealed similar results with the ELISA results. Concordantly, significantly incre-

ased NF-κB p65 immunoreactivity was detected in the cytoplasm of endometrial cells. In line with this, H-Score of NF-κB p65 in the endometrial samples obtained from RIF women was found to be significantly increased when compared to control group.

Discussion

Implantation is an inflammatory process that characterized by secretion of growth factors and cytokines including TNF, cyclooxygenase-derived prostaglandins, and NF-kB from the feto-maternal surface[8,18,19]. Both the production and release of these cytokines change in women with failed implantation. As supportive, expression levels of IFNγ in trophoblast of women suffering from recurrent abortion were found to be increased[20]. NF-κB is one of the most important mediators of inflammatory cytokines that expressed in many tissue including endometrium[15,21]. In the current study, we demonstrated for the first time that expression levels of endometrial NF-κB increased significantly in RIF subjects. Concordantly, both ELISA and immunohistochemical analysis of endometrial samples confirmed the presence of abnormal endometrial inflammation in RIF cases.

The pivotal role of inflammation in different conditions leading to infertility is well defined[2]. Concordantly, peritoneal inflammatory molecules of patients with endometriosis and tubal fluid of patients with uni or bilateral hydrosalpinges can disturb normal function of endometrium cells [2,22,23]. Aberrant secretion of inflammatory molecules might be responsible for the failed expression of receptivity genes in otherwise ''in phase'' endometrium[2,22,23]. In good agreement with above-mentioned findings, increased expression of endometrial NF-κB might be suggestive of impaired endometrial receptivity in RIF women.

The endometrium is a final destination allowing an embryo to attach receptive zone under sufficient amounts of relevant receptivity molecules[2,24]. Disturbed expression of endometrial receptivity genes and molecules during the window of implantation might be common factors in patients with implantation failure[2,7,8]. The NF-κB pathway is responsible for the regulation of DNA transcriptions, immune responses, and activation of relevant genes[15]. Previous studies[2,7,8] indicated that abnormality in NF-κB expression led to the development of implantation failure. Current study provided direct evidence for abnormally increased endometrial NF-κB expression in RIF participants. The disturbance of physiological expression pattern of NF-κB in the endometrium might contribute for the failed implantation observed in RIF women. Concordantly, investigations[15,25] demonstrated that pathological endometrial inflammation is reported to be detrimental on progesterone effect on endometrium. Truly, progesterone stimulus is essential for the decidualization and the establishment of the HOXA-10 and HOXA-11 expression in the secretory endometrium[26,27]. Collectively, by leading progesterone resistance increased NF-κB expression may impair endometrial receptivity of RIF women. Incompatible with our findings, Kalkhoven et al[28] demonstrated the existence of reciprocal antagonism between progesterone receptor and NF-κB p65 expression. Conversely, Dharmaraj et al[8] noted the positive relation between progesterone receptor and NF-κB expression supporting the critical role of this transcription factor for achieving blastocyst implantation. Moreover, Yang et al[29] reported that NF-κB action in living tissue is realized by microRNAs. Therefore, the impact of NF-κB on progesterone receptor might be mediated by microRNAs. This needs to be clarified with further researches.

Conclusions

In view of the above-mentioned facts, a remarkable increase in the expression of endometrial NF-κB1 and NF-κB p65 may predict poor reproductive outcome and inflammatory basis of RIF. Understanding the possible mechanism of actions of inflammatory events that mediated by endometrial NF-κB can help in developing new treatment agents for controlling physiological endometrial inflammation. This may lead to improving implantation success in women with RIF. In the near future, regulation of endometrial inflammation either medical agents or minimal invasive surgery can improve IVF outcomes in RIF cases. Together, our findings provided first molecular data about the existence of pathological endometrial inflammation in RIF cases.

Conflict of Interest

The authors declare no conflicts of interest.

References

1) Kalma Y, Granot I, Gnainsky Y, Or Y, Czernobilsky B, Dekel N, Barash A. Endometrial biopsy induced

gene modulation: first evidence for the expression of bladder-transmembranal uroplakin Ib in human endometrium. Fertil Steril 2009; 91: 1042-1049.

2) Celik O, Acet M, Kucuk T, Haberal ET, Acet T, Bozkurt M, Sahin L, Verit FF, Caliskan E. Surgery for benign gynecological disorders ımprove endometrium receptivity: a systematic review of the literature. Reprod Sci 2016 Jul 8. pii: 1933719116654993. [Epub ahead of print].

3) Tapia A, Gangi LM, Zegers-Hochschild F, Balmaceda J, Pommer R, Trejo L, Pacheco IM, Salvatierra AM, Henríquez S, Quezada M, Vargas M, Ríos M, Munroe DJ, Croxatto HB, Velasquez L. Differences in the endometrial transcript profile during the receptive period between women who were refractory to implantation and those who achieved pregnancy. Hum Reprod 2008; 23: 340-351.

4) Achache H, Tsafrir A, Prus D, Reich R, Revel A. Defective endometrial prostaglandin synthesis identified in patients with repeated implantation failure undergoing in vitro fertilization. Fertil Steril 2010; 94: 1271-1278.

5) Li TC, Klentzeris L, Barratt C, Warren MA, Cooke S, Cooke ID. A study of endometrial morphology in women who failed to conceive in a donor insemination programme. Br J Obstet Gynaecol 1993; 100: 935-938.

6) Ruiz-Alonso M, Blesa D, Díaz-Gimeno P, Gómez E, Fernández-Sánchez M, Carranza F, Carrera J, Vilella F, Pellicer A, Simón C. The endometrial receptivity array for diagnosis and personalized embryo transfer as a treatment for patients with repeated implantation failure. Fertil Steril 2013; 100: 818-824.

7) Cakmak H, Guzeloglu-Kayisli O, Kayisli UA, Arici A. Immune-endocrine interactions in endometriosis. Front Biosci (Elite Ed) 2009; 1: 429-443.

8) Dharmaraj N, Wang P, Carson DD. Cytokine and progesterone receptor interplay in the regulation of MUC1 gene expression. Mol Endocrinol 2010; 24: 2253-2266.

9) Luo L, Li DH, Li XP, Zhang SC, Yan CF, Wu JF, Qi YH, Zhao J. Polymorphisms in the nuclear factor kappa B gene association with recurrent embryo implantation failure. Genet Mol Res 2016; 15.

10) Lind DS, Hochwald SN, Malaty J, Rekkas S, Hebig P, Mishra G, Moldawer LL, Copeland Em 3rd, Mackay S. Nuclear factor-kappa B is upregulated in colorectal cancer. Surgery 2001; 130: 363-369.

11) Renard P, Raes M. The proinflammatory transcription factor NFkappaB: a potential target for novel therapeutical strategies. Cell Biol Toxicol 1999; 15: 341-344.

12) Chen LF, Greene WC. Shaping the nuclear action of NF-kappaB. Nat Rev Mol Cell Biol 2004; 5: 392-401.

13) Karin M, Yamamoto Y, Wang QM. The IKK NF- kappa B system: a treasure trove for drug development. Nat Rev Drug Discov 2004; 3: 17-26.

14) Hoffmann A, Baltimore D. Circuitry of nuclear factor kappaB signaling. Immunol Rev 2006; 210: 171-86.

15) Celik O, Celik E, Turkcuoglu I, Yilmaz E, Ulas M, Simsek Y, Karaer A, Celik N, Aydin NE, Ozerol I, Unlu C. Surgical removal of endometrioma decreases the NF-kB1 (p50/105) and NF-kB p65 (Rel A) expression in the eutopic endometrium during the implantation window. Reprod Sci 2013; 20: 762-770.

16) Budwit-Novotny DA, Mccarty KS, Cox EB, Soper JT, Mutch DG, Creasman WT, Flowers JL, Mccarty KS JR. Immunohistochemical analyses of estrogen receptor in endometrial adenocarcinoma using a monoclonal antibody. Cancer Res 1986; 46: 5419-5425.

17) Lessey BA. The use of integrins for the assessment of uterine receptivity. Fertil Steril 1994; 61: 812-814.

18) Carson DD, Bagchi I, Dey SK, Enders AC, Fazleabas AT, Lessey BA, Yoshinaga K. Embryo implantation. Dev Biol 2000; 223: 217-237.

19) Dey SK, Lim H, Das SK, Reese J, Paria BC, Daikoku T, Wang H. Molecular cues to implantation. Endocr Rev 2004; 25: 341-373.

20) Hill JA, Polgar K, Anderson DJ. T-helper 1-type immunity to trophoblast in women with recurrent spontaneous abortion. JAMA 1995; 273: 1933-1936.

21) Gaemers IC, Vos HL, Volders HH, Van Der Valk SW, Hilkens J. A Stat-responsive element in the promoter of the episialin/ MUC1 gene is involved in its overexpression in carcinoma cells. J Biol Chem 2001; 276: 6191-6199.

22) Seli E, Kayisli UA, Cakmak H, Bukulmez O, Bildirici I, Guzeloglu-Kayisli O, Arici A. Removal of hydrosalpinges increases endometrial leukaemia inhibitory factor (LIF) expression at the time of the implantation window. Hum Reprod 2005; 20: 3012-3017.

23) Nackley AC, Muasher SJ. The significance of hydrosalpinx in in vitro fertilization. Fertil Steril 1998; 69: 373-384.

24) Young SL, Al-Hendy A, Copland JA. Potential nonhormonal therapeutics for medical treatment of leiomyomas. Semin Reprod Med 2004; 22: 121-130.

25) Aghajanova L, Velarde MC, Giudice LC. Altered gene expression profiling in endometrium: evidence for progesterone resistance. Semin Reprod Med 2010; 28: 51-58.

26) Achache H, Revel A. Endometrial receptivity markers, the journey to successful embryo implantation. Hum Reprod Update 2006; 12: 731-746.

27) Taylor HS, Arici A, Olive D, Igarashi P. HOXA10 is expressed in response to sex steroids at the time of implantation in the human endometrium. J Clin Invest 1998; 101: 1379-1384.

28) Kalkhoven E, Wissink S, Van Der Saag PT, Van Der Burg B. Negative interaction between the RelA (p65) subunit of NF-kappaB and the progesterone receptor. J Biol Chem 1996; 271: 6217-6224.

29) Yang Y, Wang JK. The functional analysis of MicroRNAs involved in NF-κB signaling. Eur Rev Med Pharmacol Sci 2016; 20: 1764-1774.