The effect of copper intrauterine devices on the expression of mucin 1 and integrin β1 in the luteal phase endometrium

Bakırlı rahim içi araçların luteal faz endometriyumunda musin 1 ve integrin β1 ekspresyonu üzerine etkisi

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Abstract

Objective: To evaluate the effect of a copper intrauterine device on the expression of mucin- 1 (MUC1) and İntegrin β1 in the luteal phase endometrium.

Material and Methods: 25 regularly menstruating women (25-35 years) who were willing to use copper intrauterine device contraception participated in this study. Endometrial sampling via a Pipelle canulla was performed on the 24th day of their cycle and repeated three months after insertion of TCu380A IUD. Immunohistochemical staining was performed for MUC1 and integrin β1 in the endometrial sections. Staining intensity was graded under the conventional light microscope.

Results: The mean age of the study population was 32.8±5.3 years (25-35). MUC1 expression of the endometrial luminal epithelium cytoplasm and the luminal epithelium increased significantly after three months of IUD usage ($p=0.01$; $p<0.001$ respectively). Neither integrin β1 expression of endometrial luminal epithelium cytoplasma nor of the endometrial stroma changed after three months of IUD usage ($p=0.16$; $p=0.22$ respectively).

Conclusion: The increase of the embryo implantation inhibitor MUC1 synthesis may be responsible for the IUD's mechanism of action for pregnancy prevention. Integrin β1 expression of the endometrial luminal epithelium cytoplasm and stroma are not affected by the use of copper IUD. (J Turkish-German Gynecol Assoc 2009; 10: 194-8)

Key words: MUC-1, Integrin β, 1, intrauterine device, TCu380A, luteal phase endometrium

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Özet

Amaç: Bakırlı rahim içi aracın (RİA) luteal faz endometriumunda musin-1 (MUC1) ve İntegrin β1 üzerine etkilerinin incelenmesi.

Gereç ve Yöntemler: Kontraseptif yöntem olarak bakırlı rahim içi araç kullanma konusunda gönüllü olan ve düzenli adet gören (25-35 yaş arası) toplam 25 kadın bu çalışmaya katılmıştır. Siklusun 24. gününde ve TCu380A rahim içi araç uygulanmasından 3 ay sonrasında Pipelle ile endometriyal örnekleme yapılmıştır. Endometriyum kesitlerinde MUC1 ve İntegrin β1 için immünohistokimyasal boyama işlemi uygulanmıştır. Konvansiyonel ışık mikroskopu eşliğinde boyanma yoğunluğu derecelendirilmiştir.

Bulgular: Çalışma grubunun ortalama yaşı 32.8±5.3 (25-35) olarak belirlenmiştir. Üç ay süre ile RİA kullanılmasının ardından endometrial luminal epitel sitoplazması ve luminal epitel MUC1 ekspresyonunda anlamlı artış belirlenmiştir (sırasıyla $p=0.01$; $p<0.001$). Ne endometrial luminal epitel sitoplazma ne de endometrial stromada üç aylık RİA kullanılması sonrasında İntegrin β1 ekspresyonunda farklılık belirlenmemiştir (sırasıyla $p=0.16$; $p=0.22$).

Sonuç: RİA'nın gebeliği önleyici etkisinden embryo implantasyon inhibitörü olarak bilinen MUC1 ekspresyonunun artışı sorumlu olabilir. Bakırlı RİA kullanılması ile, endometrial luminal epitel sitoplazmasında ve stromasında İntegrin β1 ekspresyonunda farklılık gözlenmemiştir.

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Anahtar kelimeler: MUC-1, İntegrin β, 1, rahim içi araçlar, TCu380A, luteal faz endometriyum

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Introduction

Mucins are a family of highly glycosylated, high-molecular-weight (>250kDa) glycoproteins present on epithelial surfaces, including human endometrial epithelial cells (1). The MUC1 is an integral membrane glycoprotein with a large ectodomain containing a variable number of 20-amino acid tandem repeats, resulting in a large and highly extended structure that is both immunogenic and extensively glycosylated (2). A fraction of uterine epithelial cell mucins (30%-50%) appears to be released from the apical surface into the lumen; the remainder is presumably degraded intracellularly following endocytosis (3).

Expressed at high levels at the cell surface, MUC1 has the ability to inhibit cell-cell adhesion. This property depends on the presence of a long ectodomain and probably results from steric hindrance of receptor-ligand interactions mediated by families of adhesion receptors such as the cadherins (4). In a

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study it was shown that progesterone combined with estradiol priming induced an up-regulation of MUC1 at the receptive endometrium and during the apposition phase, the presence of a human embryo increased endometrial epithelial MUC1 (5). These findings strongly suggest that MUC1 may act as an endometrial antiadhesive molecule that must be locally removed by the human blastocyst during the adhesion phase.

Cell adhesion molecules fall into four major groups: integrins, cadherins, selectins, and members of the Ig superfamily. Integrins are transmembrane heterodimeric glycoproteins consisting of two noncovalently associated α and β subunits. Integrins are involved in cell-to-cell binding and in cell interactions with the extracellular matrix (6). The expression of integrins on the luminal surface of the endometrial epithelium changes throughout the menstrual cycle and their expression is hormonally regulated. Several studies have provided indirect evidence that integrins are potential markers of endometrial receptivity and that they participate in embryo-endometrial interactions. Recent investigations indicate that $\alpha_v\beta_3$ binds to and activates matrix metalloproteinases and plasminogen activators in the extracellular matrix (7).

The copper intrauterine device (IUD) was shown to cause a sterile inflammation and foreign body reaction in the endometrium (8). Copper ion was shown to prevent transtubal sperm and ovum migration and prevent fertilization (9). Apart from all these facts, the exact molecular mechanism of the contraceptive effect of IUDs is still not known.

In this study, we investigated the effect of copper IUD on MUC1 and Integrin β1 in the luteal phase endometrium.

Materials and Methods

The study population consisted of 25 women with at least two prior healthy term vaginal deliveries whose midluteal phase progesterone levels were ≥10 ng/mL (31.8 nmol/L) and who were willing to use copper-IUD contraception. The women were menstruating regularly (i.e. menstrual cycle varying between 28-35 days) and they were under 35 years old. The exclusion criteria were pregnancy, acute or chronic pelvic inflammatory disease, and metrorrhagia for unknown reasons, cervicitis, and dysplasia in the cervix, genital tumor, copper allergy, and usage of contraceptive pills within the previous 3 months, lactating women, hypothyroidism, hyperthyroidism, abnormalities in blood clotting and severe dysmenorrhea. Informed consent was obtained from women and the study was approved by the ethical committee of the Medical Faculty of Kocaeli University.

All patients underwent a gynecological examination and had a Papanicolaou smear taken during the previous 12 months. Menstruation period and/or quantity was asked before and three months after the IUD insertion. The biopsy specimens were taken with a Pipelle canulla without dilatation of cervix and without anesthesia. Endometrial biopsies were taken from the anterior and posterior walls of the mid-uterine cavity before and 3 months after the insertion of the IUD on cycle days 20-24. All patients at the time of IUD insertion and at the third month of IUD insertion had a progesterone level of ≥10 ng/mL (31.8 nmol/L) as an indicator of a luteal phase of an ovulatory cycle.

The endometrial specimens were washed and soaked in a graded series of ethanol and were then embedded in paraffin wax. Sections were cut to 3-5μm thickness and mounted onto adhesive coated slides (Menzel-Gloser, Süperfrost® Plus). For immunohistochemical staining sections were kept at 56^{0}C overnight and then soaked in xylene for 30 minutes. After washing with a decreasing series of ethanol, sections were washed with distilled water and phosphate-buffered saline (PBS) for 15 minutes. After the coated slides were dried in the incubator at 56^{0}C for two hours, antigen unmasking was performed for MUC1 and İntegrin β1 antibodies in the pressure cooker.

After antigen unmasking, step slides were washed with PBS and thereafter, to block endogenous peroxidase activity, the slides were incubated in 3% hydrogen peroxide for 20 minutes. Slides were washed with PBS for five minutes. Sections were then blocked with Super Block (REF:AAA 125 LOT:12232) at room temperature for 15 minutes and afterwards washed with PBS. Slides were immunostained with commercially available epitope specific rabbit antibody MUC1 (Neo-Markers Fremant, CA Cat No:RB-9222-P, USA) at 1:100 dilution for one hour at room temperature (20-25^{0}C) and were immunostained with commercially available mouse monoclonal antibody Integrin β1 (CD29) Ab-3 (Clone 29C03; same as 7F10) (Neo-Markers Fremant, CA Cat No:MS-1089-S, USA) at 1:25 dilution for two hours at room temperature (20-25^{0}C). The slides were then washed with PBS and incubated with UltraTek Anti-Polyvalent Biotinylated Antibody (REF:ABN 125, LOT:11461, Company:ScyTek Laboratories, USA) at room temperature for 25 minutes. The slides were washed with PBS again and incubated with UltraTek HRP (REF:ABL125, LOT:11460, Company:ScyTek Laboratories, USA) at room temperature for 25 minutes. The slides were washed with PBS and incubated with Ultravision Detection System Large Volume AEC Substrate System (RTU) (REF:TA-125-HA, LOT:AHA60718, Company:LabVision Fremant, CA, USA) at room temperature for 15 minutes. The sections were finally counterstained using Mayer's haematoxylin and mounted in an aqueous medium.

The slides were analyzed with a BX50 conventional light microscope (Olymphus, Tokyo, Japan) by BM at 100 and 200 magnification twice. Staining intensity was graded as; "0 = no staining", "+1 or <10% staining = weak staining", "+2 or 10-49% staining = mild staining" and "+3 or 50-100% staining = strong staining". Immunohistochemical staining in luminal and glandular epithelium cytoplasm were graded in 60 sections counting 100 cells separately at 400 magnifications.

The statistical analysis of the study data was performed using SPSS 11.5 for Windows packet programme. Paired samples t-test was used for immunostaining grade and intensity before and 3 months after the IUD insertion. Analysis of classified data was performed using the Chi-square test and Fisher's exact test. Correlation of the data was determined using the Pearson correlation test. A probability (p) value smaller than 0.05 was considered statistically significant. All values given were means (±SD) or percentage (%).

Results

The mean age of the study population was 32.8±5.3 years (25-35). The mean gravida was 2.2±1.3. Two (8%) women had one prior abortion and none had a history of infertility treatment.
Expression grade of the antibody in the luminal epithelium is presented in Table 1. MUC1 expression of endometrial luminal epithelium surface increased significantly after three months of IUD usage (p=0.01). The evaluation and grading of the MUC1 staining is presented in Figure 1.
Before IUD replacement, MUC1 expression of the luminal epithelium was present in 61±21.5 (39-92) cells while after IUD usage, it was present in 91.4±6.27 (82-98) cells. MUC1 expression of the luminal epithelium increased significantly after IUD replacement (p<0.001).
Expression grade of the antibody in the luminal epithelium and endometrial stroma is presented in Table 1. Neither Integrin β1 expression of endometrial luminal epithelium cytoplasm nor of the endometrial stroma changed after three months of IUD usage (p=0.16; p=0.22 respectively). The evaluation and grading of the integrin β1 staining is presented in Figure 2.
Before IUD replacement, Integrin β1 expression of the luminal epithelium was present in 17.6±18.7 (0-45) cells, while after IUD usage it was present in 23.4±16.2 (3-40) cells. Integrin β1 expression of the luminal epithelium did not change after IUD replacement (p=0.24). Before IUD replacement, Integrin β1 expression of the endometrial stroma was present in 2.6±2.91 (0-7) cells, while after IUD usage it was present in 9±16.2 (0-45) cells. Change of the Integrin β1 expression of the endometrial stroma was found to be insignificant after three months of IUD usage (p=0.06).

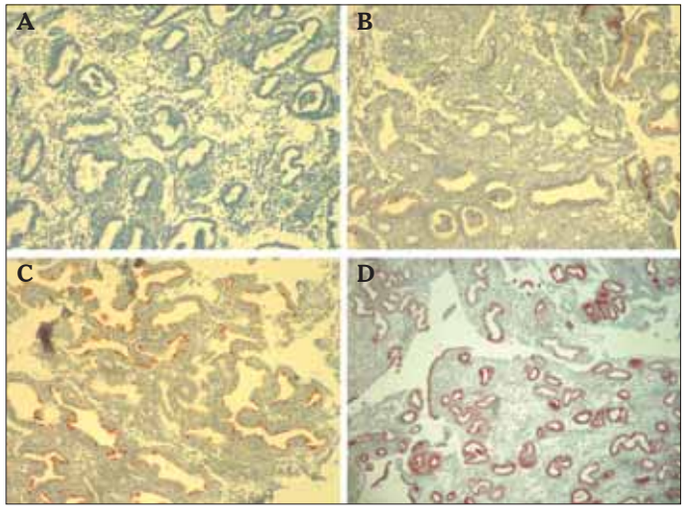

Figure 1. The evaluation and grading of MUC1 staining. Strong staining as red areas. 1a. No staining, 1b. Weak staining, 1c. Mild staining, 1d. Strong staining

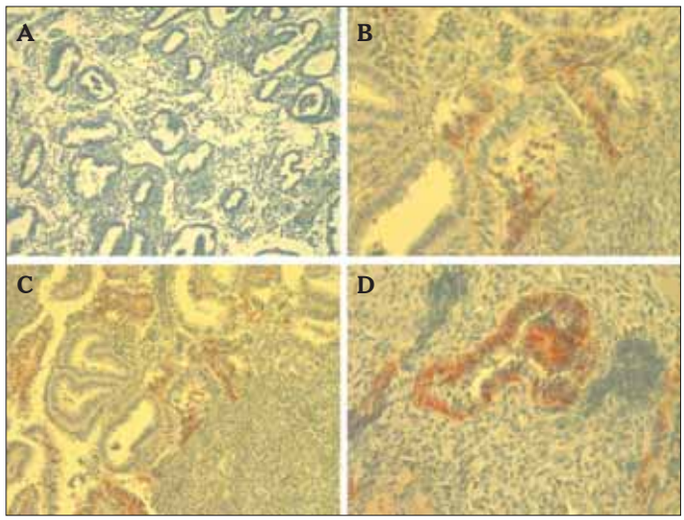

Figure 2. The evaluation and grading of integrin β1 staining. Strong staining as orange red areas 2a. No staining, 2b. Weak staining, 2c. Mild staining, 2d.

Discussion

The IUD is reported to be used throughout the world by millions of women as an effective, safe, and convenient method of preventing pregnancy. Its mechanism of action has not been elucidated completely; however, recent research has suggested that it usually prevented fertilization rather than implantation (10). Intrauterine devices (IUDs) were known to produce a local

Table 1. Expression grade of the MUC1 and Integrin β1 antibodies in luminal epithelium and endometrial stroma

Antibody	Expression Grade	Before IUD (n=25)	After IUD (n=25)	p
MUC1 immunostaining in luminal epithelium surface	0 +1 +2 +3	 3 (12%) 11 (50%) 11 (50%)	 25 (100%)	 0.01
Integrin β1 immunostaining in luminal epithelium cytoplasma	0 +1 +2 +3	 5 (20%) 11 (44%) 9 (36%)	 12 (48%) 13(52%)	 0.16
Integrin β1 immunostaining in endometrial stroma	0 +1 +2 +3	 13 (52%) 12 (48%)	 8 (32%) 14 (56%) 3 (12%)	 0.22

foreign body inflammatory reaction within the uterus (11). In a trial, women with an IUD had shown a pattern of contractility that was uncoordinated, with decreased frequency at midcycle and they concluded that uncoordinated midcycle contractions produced by the IUD might affect sperm transport (12).

Mucins are high molecular weight glycoproteins that provide a protective layer on epithelial surfaces. They are involved in cell-cell interactions, signaling, and metastasis (13). Thus far, a total of 19 human mucins have been identified, and more are possibly awaiting discovery. The MUC1 mucin is a transmembrane glycoprotein with an extracellular domain consisting of a variable number of highly conserved tandem repeats of 20 amino acids (14). Over expression of MUC1 by cultured cells inhibits their aggregation, possibly because of its large, extended, and rigid external structure (15). This mucin is ubiquitously expressed in all reproductive tract epithelial tissues, including the fallopian tubes, uterus, endocervix, and vagina, and its immunoreactivity is observed at the apical cell surface at all levels of the fallopian tubes and throughout the secretory phase of the menstrual cycle (16, 17).

It has been widely accepted that IUDs work primarily by inhibiting fertilization due to direct toxicity (18). A systematic review on the mechanisms of action of IUDs showed that pre- and post-fertilization effects contribute to efficacy (19). An inflammatory reaction within the endometrium may have an anti-implantation effect but an IUD is not an abortifacient (20). The anti-implantation effect may be due to increased synthesis of MUC1 in the endometrium. In our study, we demonstrated that MUC1 expression increases after IUD use for a time and in our opinion, mucin is one of the major determinants responsible for the anti-implantation effect. The role of mucins have been investigated in implantation of embryos (21, 22). Large-molecular-weight mucin glycoproteins such as MUC1 are present at the apical surface of the uterine epithelium and they are antiadhesive and also appear to represent a barrier to embryo attachment. This data also suggests that increased mucin expression causes an antiadhesive barrier to embryo attachment.

In our study, we demonstrated that IUD has no effect on the endometrial expression of Integrin β1. Therefore, IUD may act via other integrin subunits. Subunit β_3 has been possibly implicated in early embryo-endometrial interactions during human blastocyst implantation. The immunohistochemical expression of αvβ3 in midluteal endometrial samples has been proven to be a marker of endometrial receptivity (6). In another study investigating the expression of α_4 and β_3 integrin subunit levels in the endometrium of healthy women and copper intrauterine device (IUD) T200 users, no difference was found in α4 integrin expression between IUD users and controls in both luminal and glandular epithelium and proportionately, significantly fewer women using copper IUD had positive $\alpha_v\beta_3$ immunoreactivity in the glandular epithelium of mid-secretory endometrium (23).

The balance of evidence suggests that the use of an IUD does not affect return to fertility. The increase of the suggested embryo implantation inhibitor MUC1 synthesis may be one of the mechanisms of action of the IUD's effect in pregnancy prevention. Integrin β1 expression of the endometrial luminal epithelium cytoplasm and stroma are not affected with the use of the copper IUD.

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