Comparison of the Effects of Copper T and Levonorgestrel IUD on Proteoglycan Composition of Cervical Mucus

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OBJECTIVE: The aim of this study was to determine the effects of Copper T and Levonorgestrel IUD (LNG-IUD) on the proteoglycan composition of cervical mucus.

STUDY DESIGN: This was a cross-sectional study that was conducted at a gynecology outpatient clinic of a University hospital for a six month period. The study participants were between 18-50 years old, using either Copper T or LNG-IUD for contraception. Cervical mucus samples were collected 7 days after the menstrual bleeding and the levels of hyaluronan (HA), heparin sulfate (HS) and sulfated-glycosaminoglycans (s-GAG) were evaluated.

RESULTS: The levels of HA, HS and s-GAG in the Copper-T and LNG-IUD groups were higher than those of the controls. However, the difference was statistically significant only for s-GAG ($p<0.001$).

CONCLUSION: Copper T and Levonorgestrel intrauterine contraceptive devices have significant effects on proteoglycan component of cervical mucus.

Keywords: Cervical mucus, Copper T IUD, Levonorgestrel IUD

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Introduction

Cervical mucus covers the epithelial surface of the cervix that is a passageway from the vagina to the peritoneal cavity. Cervical mucus protects the cell lining from micro-organismal attacks and sperm penetration. Furthermore cervical mucus secretion is altered in accordance with hormonal stimuli.[1] However, there is limited data about the impact of different IUDs on the biochemical composition of the cervical mucus.

The effects of contraceptive devices on the composition of cervical mucus were investigated in a previous study. The trace elements were elevated in Copper T IUD users, which is spermicidal.[2] Levonorgestrel (LNG) IUD induced thickening of cervical mucus that inhibited the migration of the spermatozoa.[3]

Glycosaminoglycans (GAG) are among the components of the extracellular matrix and contain disaccharide units that are acetylated hexosamine and uronic acid repeats.[4] Heparin sulfate (HS), hyaluronan (HA), dermatan sulfate, keratin sulfate and chondroitin sulfate are among the major constituents of sulfated GAGs. Sulfated (s-) GAG form complexes with proteins. Proteoglycans contribute to cell-cell interaction events during inflammatory reactions, immune response, tissue injury and wound healing.[4]

Cell surface GAGs interact with growth factors and exert important effects on cell adhesion, migration, proliferation and differentiation.[5]

The aim of this study is to determine the effects of two different kinds of IUDs on the composition of cervical mucus proteoglycans and to compare the quantitative levels of s-GAG, HS and HA in IUD users to those of non-users.

Material and Method

This was a cross-sectional study that was conducted at a gynecology outpatient clinic of a university hospital for a six month period. The study protocol was approved by the Institutional Review Board and each participant gave written informed consent.

The inclusion criteria were use of contraceptive Copper T or LNG-IUD and being between 18-50 years of age. Menopause, desire for pregnancy, lactation, acute vaginitis, endometritis, pelvic inflammatory diseases, systemic diseases (diabetes mellitus, hypertension, etc.), uterine anomalies, abnormal cervical cytology, and smoking constituted the exclusion criteria.

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The participants were systemically and gynecologically evaluated. Speculum examination was performed for detection of vaginal discharge or cervicitis. Participants were treated with metronidazole 500 mg orally twice a day for 7 days prior to cervical mucus collection if infection was detected. Mucus sample collection coincided with 7 days postmenstrual bleeding.

Samples were centrifuged and the supernatants were stored at -20° C until testing. Samples were thawed and analyzed by enzyme-linked immunosorbent assay (ELISA) using commercially available antibodies to HA, HS and s-GAG (Eastbiopharm, Hangzhou, China) along with suitable controls. Inter- and intra-assay variations were reported to be <10% and <12%, respectively. The lower limits of sensitivities were reported to be 1.02ng/ml for HA, 1.12ng/ml for HS and 0.09mg/L for s-GAG.

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) version 17 (Chicago, IL, USA). The data and variables were tested with Kolmogorov-Smirnov test with Lilliefor's significance correction for normal distribution. One-Way ANOVA test with Bonferroni correction was performed for the comparison of the groups. A P value of <0.05 was accepted as statistically significant. Data was given as mean ± standard deviation or number of cases and percentages.

Results

Participants were separated into three groups of 20 subjects: group 1 were LNG-IUD users, group 2 were Copper T IUD users, and group 3 were controls not on contraceptives.

Mean age, gravida and parity were 41.40±6.27 years, 2.5 ±1.1 and 2.46 ± 0.9, respectively. There was no statistically significant difference between the groups in respect of demographic characteristics (p>0.05) (Table 1). The proteoglycan levels were determined by ELISA. Their means were 40.91± 36.24 for s-GAG, 136.21±107.01 for HS, and 134.27± 128.85 for HA. The levels of HA, HS and s-GAG in groups 1 and 2 were higher than those of group 3. But the difference was statistically significant only for s-GAG (p<0.001) (Figure 1). The difference for HS and HA levels was statistically insignificant levels (p>0.05) (Figure 2,3) (Table 1).

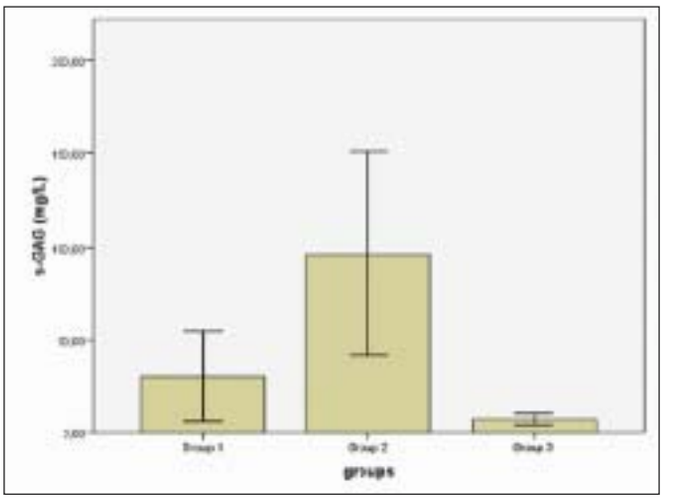

Figure 1: Sulfated glycosaminoglycan(s-GAG) levels in study groups.

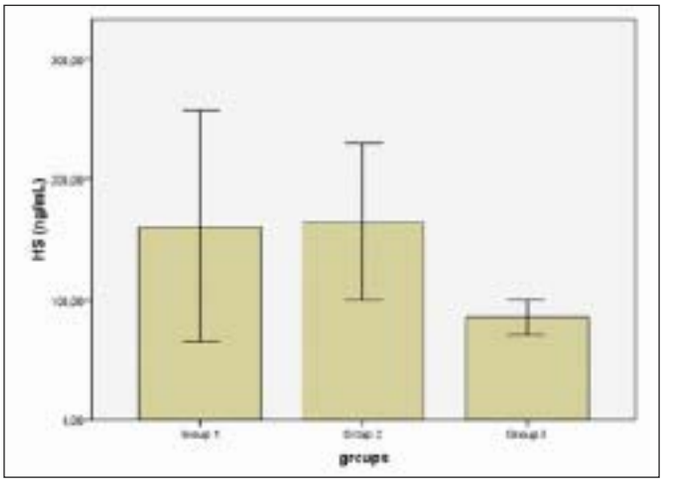

Figure 2: Heparan sulfate (HS) levels in study groups

Table 1: Comparison of demographic characteristics and proteoglycan levels

Parameters	Group 1 (n: 20)	Group 2 (n: 20)	Group 3 (n: 20)	P value
Age (years)	39.7±8.4	39.3±3.9	45.0±2.9	0.058
Gravida (number)	2.1±0.9	2.4±0.5	2.9±1.4	0.238
Parity (number)	2.1±0.8	2.4±0.4	2.9±1.3	0.169
Body mass index (kg/m^2)	24.6±1.2	25.1±0.8	24.8±0.9	0.256
Menstrual cycle length (days)	27.6±4.2	28.2±3.7	27.1±4.5	0.153
s-GAG	30.33±38.38	96.20±70.95	7.21±4.70	<0.001*
HA	167.93±197.81	153.86±62.95	81.51±22.14	0.244
HS	161.13±13	164.77±84.00	85.67±21.73	0.155

**Group 2 was significantly different from groups 1 and 3 (p<0.001)*
Abbreviations: s-GAG: Sulfated-Glycosaminoglycan, HA: Hyaluronan, HS: Heparin sulfate

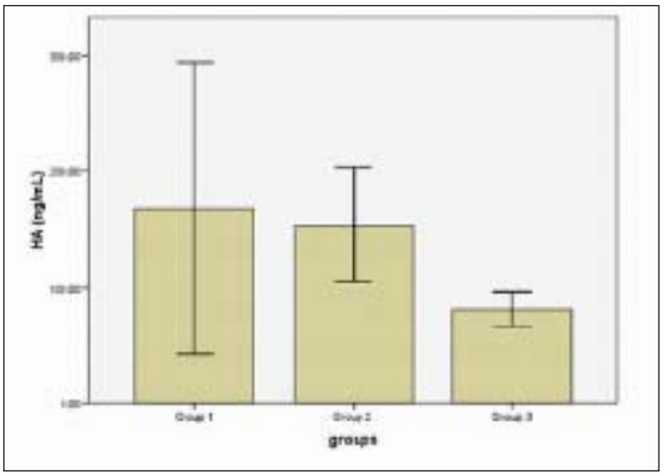

Figure 3: Hyaluronan (HA) levels in study groups

Discussion

Intrauterine contraceptive devices have worldwide acceptability for their long-lasting effects and high reliability. Copper T IUD and LNG-IUD are the most preferred devices for contraception. Their contraceptive mechanisms are different. Copper T IUD has local inflammatory and spermicidal effects while LNG-IUD causes atrophy and decasualization. Additional local and systemic biochemical reactions and physical effects enhance their contraceptive effects. IUD induced cervical mucus alterations have important contributions to their contraceptive effects.[6]

Our results showed that proteoglycan levels in cervical mucus were altered by IUDs in comparison to the control group. Even though sulfated-GAG levels were elevated in both IUD groups in comparison to the controls, the difference was significant only in Copper T IUD users. The HS and HA levels were also elevated in each IUD group though this was insignificant.

Mucus is a protection barrier for epithelial surfaces from hostiles like microorganisms. Cervical mucus protects the female genital tract from infections and sperm migration. Components of the cervical mucus include water (>90%), proteins, electrolytes, glycoproteins and proteoglycans.[7] The composition of cervical mucus can be modified by the hormonal stimuli and synthetic polymers.[8]

Pathophysiologic cervical mucus alterations have been described in several reports. Vaginal infections, endocrine disorders and malignancies are some of the pathological causes.[9,10] In addition, oral contraceptives and hormonal drugs cause changes in cervical mucus.[11,12] Recently, environmental factors were evaluated for their effect on the microstructure of cervical mucus. Exposure to nanoparticles was found to disrupt the mucus barriers.[13]

Cervical mucus viscoelasticity changes during the menstrual period, which is regulated by steroid hormones. Viscoelasticity increase in the mid cycle and decrease in the luteal phase because of the corresponding changes in the levels of glycoprotein and water contents of the cervical secretions.[14] During ovulation, the viscosity of cervical mucus decreases.[3] Andersch-Björkman et al evaluated healthy women during the menstrual period and found that the protein content of the mucus is constant during the menstrual period but glycosylation in the cervical mucus increased during the ovulation period.[15] The modifications of cervical mucus due to oral or systemic administration of exogenous progestin were investigated previously.[16] Hormonal contraception makes the cervical mucus scanty, thick and highly viscous.[12] Lewis et al compared the mid cycle cervical mucus quality of LNG-IUD users to hormone free matching controls and found the quality of the mucus to be "poor" and its effect as preventive on sperm penetration in the former.[17]

IUDs influence the composition of cervical mucus. Jonsson et al reported that the mucin, albumin and IgG content of cervical mucus increased at Copper-IUD users but not affected at LNG-IUD users.[6] Increased levels of cytokines in the cervical mucus of Copper T IUD users as a result of inflammatory reaction was shown by Shobokshi et al.[18]

Hyaluronan is an immune modulator and activates the pro-inflammatory immune process as a response to injury or infection. In a previous study, elevated levels of hyaluronan were demonstrated in women with recurrent vulvo-vaginitis.[19] Elevated levels of HA was also found in the amniotic fluid as a component of pro-inflammatory modulation of immune response during pregnancy.[20]

Heparan sulfate is a component of cell surface and extracellular matrix. HS interacts with most growth factors, cytokines, chemokines and adhesion molecules, and modulates the immune response.[20] HS activates the endothelial cells and increases leucocyte adhesion relevant to inflammatory reaction.[21]

In conclusion; intrauterine contraceptive devices have significant effects on the glycosaminoglycan components of cervical mucus. Furthermore, Copper T- IUD alters s-GAG levels of cervical mucus which could be responsible for the tendency to inflammatory reaction. This study has a number of limitations. Firstly, the small sample size is insufficient for drawing definitive conclusions. Secondly, the absence of data concerning the glycosaminoglycan levels before insertion of IUD is another limitation. Thirdly, the cervical inflammatory cytokine levels were not evaluated in the trial. This small scale study must be performed on larger groups and the proteoglycan levels should be determined in parallel with inflammatory cytokines.

Cooper T ve Levonorgestrelli Rahim İçi Araçların Servikal Mukus Proteoglikan Yapısı Üzerine Etkilerinin Karşılaştırılması

AMAÇ: Bu çalışmanın amacı Cooper T ve Levonorgestrelli Rahim içi araçların (LNG-IUD) servikal mukus proteoglikan yapısı üzerine etkisinin değerlendirilmesidir.

GEREÇ VE YÖNTEM: Bu kesitsel çalışma bir üniversite hastanesi jinekoloji polikliniğinde altı ay süre ile yürütülmüştür. Dahil edilme kriterleri 18-50 yaş arasında olmak ve kontraseptif yöntem olarak Copper T ya da LNG-IUD kullanmaktır. Mukus örnekleri menstural kanamadan 7 gün sonra elde edilmiş ve servikal mukusta Hyaluronan (HA), Heparin Sulfat (HS) ve sulfatlanmış glikozaminoglikan (s-GAG) seviyeleri değerlendirilmiştir.

BULGULAR: Copper T ve LNG-IUD gruplarında HA, HS ve s-GAG seviyeleri kontrol grubundan daha yüksektir. Ancak fark sadece s-GAG için istatistiksel olarak anlamlıdır (p<0,001).

SONUÇ: Bakırlı ve levonorgestrelli rahim içi kontraseptif araçların servikal mukusun proteoglikan içeriği üzerinde önemli etkileri vardır.

Anahtar Kelimeler: Servikal mukus, Bakırlı RİA, Levonorgestrelli RİA

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