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EFFECT OF HEPARIN AND CLEXANE IN COMBINATION WITH ANTIBIOTICS AND CONTRYCAL ON DNA METHYLATION OF LEUKOCYTES IN WOMEN WITH GENITAL INFECTIONS AND MISCARRIAGE

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XULOSA

Maqsad. *Geparin, kleksan, antibiotiklar va kontrikalni o‘z ichiga olgan pregravidar terapiya rejimlarining genital infeksiyalar va homila tushish holatlari kuzatilgan ayollarda DNK-metiltransferaza 1 (DNMT1) va 5-metil-2-dezoksitsitidin (5-mdC) darajasiga ta’sirini baholash.*

Material va usullar. *Homiladorlikkacha va birinchi trimestr davomida pregravidar davolash (PMG – kleksan; FNG – geparin; antibiotiklar va kontrikal) fonida ayollarning leykotsitlaridagi epigenetik markerlar darajasi tahlil qilindi.*

Natijalar. *Sog‘lom va faqat PMG olgan ayollarda epigenetik faollikning fiziologik oshishi kuzatildi. PMG antibiotiklar va/yoki kontrikal bilan qo‘llanilganda epigenetik markerlarning dastlabki past darajalari va to‘liq tiklanmaganligi aniqlandi. FNG, ayniqsa kombinirlangan davolashda, epigenetik faollikka susaytiruvchi ta’sir ko‘rsatdi. DNMT1 va 5-mdC ning past darajalari FNG, antibiotik va kontrikal bilan davolangan bemorlarda qayd etildi.*

Xulosa. *Geparin va yallig‘lanishga qarshi vositalarni o‘z ichiga olgan pregra-vidar terapiya immun tizimdagi epigenetik jarayonlarga uzoq muddatli ta’sir ko‘rsatishi mumkin. Bu holat genital infeksiyalar va trombofilik buzilishlarga ega ayollarda homiladorlikni rejalashtirishda inobatga olinishi lozim.*

Kalit so‘zlar: *geparin, kleksan, kontrikal, genital infeksiyalar, DNK-metillanishi, homiladorlikning erta muddati, homila yo‘qotilishi.*

РЕЗЮМЕ

Цель. *Оценить влияние различных режимов терапии (гепарин, клексан, антибиотики, контрикал) на уровни ДНК-метилтрансферазы 1 (DNMT1) и 5-метил-2-дезоксицитидина (5-mdC) у женщин с генитальными инфекциями и невынашиванием беременности.*

Материал и методы. *Исследованы уровни эпигенетических маркеров в лейкоцитах у женщин до беременности и в I триместре на фоне прегравидарных схем лечения: НМГ (клексан), НФГ (гепарин), антибиотики, контрикал.*

Результаты. *У здоровых и получавших только НМГ женщин, отмечалось физиологическое повышение эпигенетической активности. При сочетании НМГ с антибиотиками и/или контрикалом наблюдалось значительное исходное снижение маркеров с неполным восстановлением. НФГ, особенно в сочетании с антибиотиками и контрикалом, оказывал ингибирующее влияние на эпигенетические параметры (DNMT1 и 5-mdC).*

Вывод. *Прегравидарная терапия с применением гепаринов и противовос-палительных препаратов оказывает долговременное влияние на эпигенетические процессы иммунной системы, что важно при планировании беременности у женщин с воспалительными и тромбофилическими нарушениями.*

Ключевые слова: *гепарин, клексан, контрикал, генитальные инфекции, метилирование ДНК, ранние сроки беременности, невынашивание беременности.*

During pregnancy, the maternal immune system undergoes temporary changes, regulated in part by epigenetic mechanisms such as DNA methylation, histone modification, and non-coding RNAs [1]. DNA methylation is crucial for extraembryonic development, particularly trophoblast function, and its disruption impairs endometrial receptivity, contributing to miscarriage [4,6]. Prenatal infections can alter DNA methylation patterns, with virus-like immune responses during early and late gestation showing distinct epigenetic effects [5]. Inflammatory cytokines may induce fetal epigenetic reprogramming, potentially leading to neurodevelopmental disorders [7]. Understanding these epigenetic processes may aid in identifying biomarkers of pregnancy risk [5]. Heparin, known for its anti-inflammatory effects through cytokine interaction and neutralization [2], may also influence DNA methylation [3].

THE AIM OF THE STUDY was to investigate the effect of heparin and clexane in combination with antibiotics and contrykal on DNA methylation of leukocytes in women with genital infections and miscarriages.

MATERIAL AND METHODS

The study included 97 women of reproductive age with genital infections, divided into 7 groups according to clinical and anamnestic criteria, including a history of miscarriages (<12 weeks) and various preconception ther-

apy regimens. The study was approved by the Ministry of Health of the Republic of Uzbekistan, and all participants signed an informed consent form. Control: 19 women with infections and miscarriages (without special therapy). Low-molecular-weight heparin (LMWH - Clexane): 16 women (20 mg/day, subcutaneously, 15 days). LMWH + antibiotics (clarithromycin, doxycycline): 13 women (15 days). Complex (LMWH + Contrycal + antibiotics): 12 women (15 days). Unfractionated heparin (UFH): 14 women (5000 IU 2x/day, 10 days). UFH + antibiotics: 12 women (10 days). UFG + Contrycal + antibiotics: 11 women (10 days).

At the pre-pregnancy preparation stage, as well as at 6 and 12 weeks of pregnancy, the levels of 5-methyl-2'-deoxycytidine (5-mdC) and DNA methyltransferase 1 (DNMT1) were determined using the ELISA method. Methylation at the C5 position of the 2'-deoxycytidine molecule leads to the formation of 5-mdC, whose concentration reflects the level of DNA methylation. Quantitative determination of 5-mdC in the supernatants of washed, hemolyzed leukocytes (isolated using Ficol-Verografin) was performed using an ELISA kit (BCM Diagnostics, USA). DNMT1 activity was determined in the same samples using a commercial ELISA kit (Human, Germany). Statistical data processing was performed using the Student's t-test, with a significance level of $p < 0.05$.

RESULTS AND DISCUSSION

In Group 1, baseline DNA methyltransferase 1 (DNMT1) levels were 61.2 ± 5.8 nM/ml, increasing slightly by week 6 (74.6 ± 7.1 nM/ml; $p>0.05$) and significantly by week 12 (83.5 ± 7.9 nM/ml; $p<0.05$). Group 2 (pre-pregnancy Clexane) showed a comparable baseline (56.4 ± 5.7 nM/ml; $p>0.05$ vs. Group 1), with non-significant rises to 67.5 ± 6.8 and 75.3 ± 7.6 nM/ml at weeks 6 and 12, respectively. In Group 3, baseline DNMT1 was lower (43.5 ± 3.9 nM/ml), increasing significantly by week 6 (51.4 ± 4.7 nM/ml; $p<0.05$) and further by week 12 (64.5 ± 6.1 nM/ml; $p<0.05$), yet still slightly below Groups 1 and 2 ($p>0.05$). Group 4 had the lowest baseline (32.1 ± 2.8 nM/ml; $p<0.001$ vs. Groups 1–2), with significant increases by week 6 (42.6 ± 3.9 nM/ml; $p<0.05$) and week 12 (53.2 ± 4.9 nM/ml; $p<0.001$), but values remained significantly lower than in Groups 1 and 2 ($p<0.05$) (Fig. A). In Group 5, pre-pregnancy DNMT1 levels were 49.3 ± 4.8 nM/ml, slightly below those of Groups 1 and 2 ($p>0.05$). Levels rose to 58.2 ± 5.9 nM/ml at week 6 and 64.7 ± 6.5 nM/ml at week 12, without statistically significant differences compared to baseline or to Groups 1 and 2 ($p>0.05$) (Fig. A).

In Group 6, DNMT1 levels were 31.6 ± 2.9 nM/ml pre-pregnancy, significantly lower than in Groups 1, 2 ($p<0.01$), and 5 ($p<0.05$). Levels rose to 42.3 ± 3.8 nM/ml at week 6 and 46.3 ± 4.4 nM/ml at week 12 ($p<0.05$ vs. baseline), yet remained significantly below those of Groups 1, 2 ($p<0.01$), and 5 ($p<0.05$). In Group 7, DNMT1 was 26.1 ± 2.4 nM/ml - lower than in Groups 1, 2, and 5 ($p<0.001$) (Fig. A).

At week , the level rose to 30.5 ± 2.7 nM/ml with no significant change from baseline ($p>0.05$), and by week 12 reached 38.9 ± 3.5 nM/ml ($p<0.05$ vs. baseline), but remained significantly lower than in Groups 1, 2, and 5 ($p<0.001$) (Fig. A).

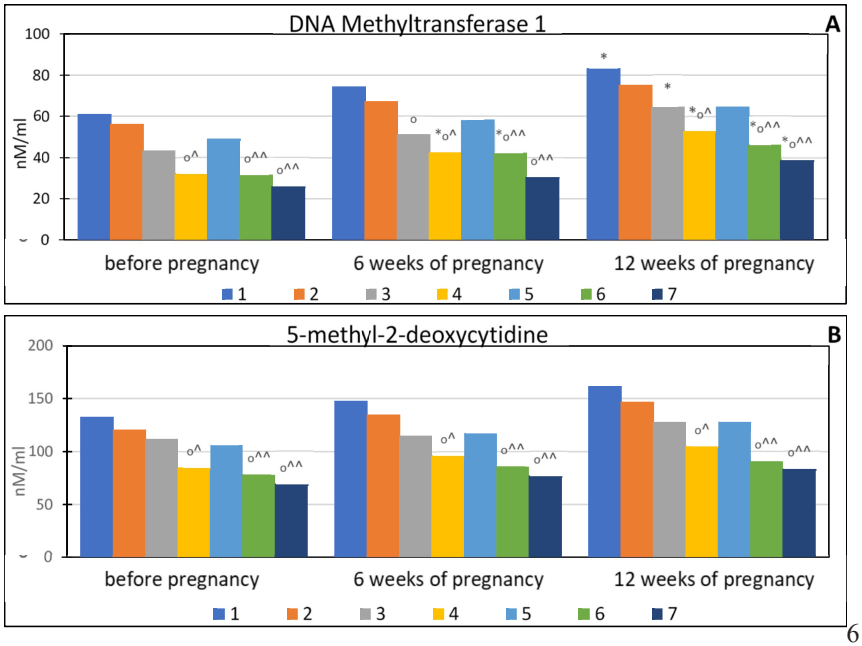
In Group 1, the level of 5-methyl-2-deoxycytidine rose from 133 ± 11.7 ng/ml pre-pregnancy to 148 ± 13.5 ng/ml at 6 weeks and 162 ± 14.9 ng/ml at 12 weeks ($p>0.05$). Group 2 showed a similar trend: from 121 ± 11.9 ng/ml to 135 ± 14.2 ng/ml and 147 ± 15.4 ng/ml, respectively ($p>0.05$ vs. Group 1). In Group 3, values increased slightly from 112 ± 10.4 ng/ml to 115 ± 11.3 ng/ml at 6 weeks and 128 ± 12.5 ng/ml at 12 weeks, remaining lower than in Groups 1 and 2 ($p>0.05$). Group 4 had significantly lower baseline levels (85 ± 8.2 ng/ml; $p<0.05$ vs. Groups 1 and 2), which rose to 96 ± 9.5 ng/ml at 6 weeks ($p<0.05$) and to 105 ± 9.7 ng/ml at 12 weeks ($p>0.05$), still remaining significantly lower than in Groups 1 and 2 ($p<0.05$) (Fig. B). In Group 5, the pre-pregnancy level of 5-methyl-2'-deoxycytidine (5-mdC) was 106 ± 9.8 ng/ml, with no significant differences compared to Groups 1 and 2 ($p>0.05$). A moderate increase was observed at 6 weeks (117 ± 11.3 ng/ml) and at 12 weeks (128 ± 13.6 ng/ml) ($p>0.05$). In Group 6, the baseline level was lower (78.3 ± 7.4 ng/ml; $p<0.01$), showing a slight rise to 86.1 ± 8.3 ng/ml at 6 weeks and to 91 ± 8.9 ng/ml at 12 weeks, remaining significantly lower than in Groups 1, 2, and 5 ($p<0.05$). In Group 7, the pre-pregnancy level was the lowest (69.6 ± 6.5 ng/ml; $p<0.001$ vs. Groups 1–2; $p<0.01$ vs. Group 5), increasing to 77.2 ± 7.4 ng/ml at 6 weeks and 83.5 ± 8.1 ng/ml at 12 weeks, but still significantly lower than in Groups 1, 2, and 5 ($p<0.001$–0.05) (see Fig. B).

In Groups 3 and 4 (LMWH with antibiotics ± Contrycal), baseline DNMT1 and 5-mdC levels were significantly lower than in Groups 1 and 2, suggesting pre-pregnancy epigenetic suppression. Although levels increased during pregnancy, particularly in Group 4, they remained lower than in untreated patients. In Groups 6 and 7 (UFH-treated), initial suppression persisted through week 12. UFH showed a stronger inhibitory effect on epigenetic markers than LMWH, especially in combination therapy ($p<0.05$–0.001).

CONCLUSIONS

Physiological increases in epigenetic activity (DNMT1 and 5-mdC) were observed in healthy women and those receiving LMWH without antibiotics, indicating normal regulation during early pregnancy. The addition of antibiotics and/or Contrycal to LMWH was associated with reduced baseline levels of epigenetic markers and their incomplete recovery. UFH, particularly in combination with anti-inflammatory therapy, had a more pronounced suppressive effect on epigenetic indicators. The most significant inhibition was noted with UFH + antibiotics + Contrycal, suggesting systemic effects of the therapy. These findings indicate that preconception anticoagulant and anti-inflammatory treatment may have long-term impacts on the epigenetic state, which should

be considered when planning pregnancy, especially in women with immune-inflammatory or thrombophilic disorders.



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Changes in the studied indicators in the blood of the women of the examined groups.

Note: 1- control group; 2 – women who received pregravidary LMWH – xlexan; 3- women who received pregravidary LMWH – clexane + antibiotic; 4- women who received pregravidary LMWH + contrykal +antibiotics; 5 - women who received pregravidary UFH. 6 – women who received pre-gravidar UFH + antibiotic; 7 – women who received pre-gravidar UFH + contrykal + antibiotic.

*- significantly different values compared to pre-pregnancy values.
o - significantly different values compared to group 1 values.
+ - significantly different values compared to group 6 weeks of pregnancy values.
^ - significantly different values compared to group 2 values.
^^ - significantly different values compared to group 5.

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