MEDICAL SCIENCES

INFLUENCE OF GENITAL INFECTIONS ON LEUCOCYTE DNA METHYLATION IN EARLY PREGNANCY MISCARRIAGE

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Abstract

This study examined the impact of genital infections on leukocyte DNA methylation in women with early miscarriage. It was concluded that in women with full-term pregnancies and full-term deliveries who had genital infections before pregnancy, a balanced ratio of proinflammatory (TNF-α, IL-1β) and anti-inflammatory (IL-10) cytokines, as well as balanced MMP/IMMP levels and moderate epigenetic changes, contribute to effective immune regulation, which facilitates a favorable pregnancy even in the presence of infection. In women who had miscarriages at 12 weeks of gestation and also had genital infections before pregnancy, a pronounced cytokine imbalance (high TNF-α, IL-1β; low IL-10) indicates excessive inflammation and the risk of complications associated with miscarriages. Significant increases in MMP and decreases in IMMP can disrupt tissue integrity, worsening conditions for pregnancy. Increased DNA methylation (elevated DNMT1 and 5-methyl-2'-deoxycytidine) indicates epigenetic abnormalities that potentially impact immune adaptation and reproductive function.

Keywords: interleukins, proteases, protease inhibitors, DNA methyltransferase 1, early pregnancy, genital infections.

Introduction

Bacteria induce changes in DNA and histone methylation patterns in the host by secreting bacterial effector proteins or hijacking host methyltransferases via bacterial structures and molecular stimuli [5]. These methylation changes affect signaling pathways, transcription factors, chromatin structure, host receptors, and microRNAs. This leads to altered expression of genes involved in the immune response, allowing immune evasion, bacterial survival, and replication within the host cell. Ultimately, these epigenetic changes facilitate effective infection [2]. Research on compounds targeting methyltransferases shows clinical relevance in the treatment of diseases in which epigenetic patterns are disrupted. Research into epigenetics and methyltransferase inhibitors in infection may help better understand the molecular mechanisms of infection. Specific chemical inhibitors may help answer these questions [7]. This may facilitate the identification of new treatment strategies that will ultimately reduce antibiotic use and help combat growing antimicrobial resistance. This area remains understudied, and further research is needed to examine the potential and actual impact of epipreparations on infection [1].

Pregnancy provides an exceptional platform for studying stress and stress responses under normal conditions and during pathogenic infection, where epigenetic regulation plays a key role in determining gestational development. A better understanding of certain epigenetic mechanisms, such as DNA methylation, post-transcriptional modification of histones, and regulatory non-coding RNAs, may help us identify biomarkers for pregnancy-related risk factors or disease risk [8]. Epigenetic alteration can also trigger inflammatory responses, such as autoimmune diseases or allergic reactions in healthy individuals. Prenatal exposure to immune factors can often be a significant factor, causing epigenetic modification and leading to the development of brain disorders in newborns [4]. Pathogenic infections (bacterial, viral, and fungal) can have a significant impact on the overall well-being of the mother and fetus. Significant changes in the cytokine profile (INF-γ, TNF-α, TGF-β, and interleukins) and epigenetic regulation during infection during pregnancy have been shown to cause adverse pregnancy outcomes [3]. Much more in-depth knowledge is required to understand the factors causing changes in epigenetic regulatory mechanisms during pregnancy under infectious and normal conditions. This may be useful for determining the phenotypes of offspring and preventing or reducing the risk of health hazards to future offspring. Detailed information in this regard may be crucial for understanding pregnancy, the immune paradigm, and other emerging complications and will be useful for taking certain basic measures to minimize the impact of infection and any other factors during pregnancy [6].

Study Objective

To study the impact of genital infections on changes in leukocyte DNA methylation in women with early pregnancy loss.

Materials and Methods

Seventy-four women were examined, divided into three groups. Group 1 included 29 women who had had a full-term pregnancy and full-term birth, and who had no history of genital infections before pregnancy. Group 2 included 25 women who had had a full-term pregnancy and full-term birth but had genital infections before pregnancy. Group 3 included 22 women who had miscarriages at 12 weeks of pregnancy and also had genital infections before pregnancy.

Blood samples from women were analyzed using ELISA before, 6, and 12 weeks of pregnancy for proinflammatory markers (interleukin-1β (IL-1) and tumor necrosis factor-α (TNF-α)), and anti-inflammatory markers (interleukin-10). Matrix metalloproteinases (MMP-9) and protease inhibitors (matrix metalloproteinase inhibitor-1).

Methylation occurs at the C5 position of the 2'-deoxycytidine molecule, forming the methylated base 5-methyl-2'-deoxycytidine. Therefore, changes in DNA methylation levels can be quantified by measuring the concentration of 5-methyl-2'-deoxycytidine. Therefore, changes in DNA methylation levels in the supernatant of washed hemolyzed leukocytes isolated in fecol-verografin were determined based on the concentration of 5-methyl-2'-deoxycytidine using an ELISA method. DNA methyltransferase 1 activity was also determined in the supernatant of washed hemolyzed lymphocytes isolated in fecol-verografin using an ELISA kit.

Statistical analysis was performed using the analysis of variation statistics, calculating mean values and their standard errors, and determining the Student-Fisher difference reliability coefficient (t). Differences were considered statistically significant at $p < 0,05$.

Results and Discussion

The studies revealed that women in the second group had significantly higher TNF-α blood levels before pregnancy than women in the first group, by 1,8 times. At 6 weeks of pregnancy, this level in women in the second group was 1,4 times higher than before pregnancy and significantly higher than similar results in the first group. At 12 weeks of pregnancy, TNF-α levels in group 2 did not change significantly compared to week 6, but were 1,9 times higher than before pregnancy levels and 1,8 times higher than those in group 1. In women in the third group, TNF-α levels before pregnancy were significantly higher than those in the first group (4,0 times) and the second group (2,2 times). At 6 weeks of pregnancy, this level in women in the third group was 2,1 times higher than before pregnancy levels, 5,5 times higher than those in the first group, and 3,3 times higher than those in the second group. At 12 weeks of pregnancy, significant changes were also observed in the third group, with TNF-α levels 3,0 times higher than pre-pregnancy levels, 6,7 times higher than in the first group, and 3,7 times higher than in the second group.

The IL-1β study revealed changes similar to those found for TNF-α. Women in the second group demonstrated significantly higher and significantly different results both before pregnancy and at 6 and 12 weeks of pregnancy, compared to women in the first group. Furthermore, these values were also significantly and significantly higher compared to pre-pregnancy levels in the same group. The third group also recorded higher levels of this parameter before pregnancy, as well as at 6 and 12 weeks of pregnancy, confirming the significant differences in the results of the third group compared to the first and second groups. Also, as a result of the studies, it was established that the level of interleukin-10 (IL-10) in the blood of women in the second group pregnancy was 1,2 times higher than in women of the first group, but the difference did not reach statistical significance. At 6 weeks of pregnancy, the level of IL-10 in the blood of women of the second group decreased, reaching 1,3 times lower than the values before pregnancy, while it was slightly higher than in group 1. At 12 weeks of pregnancy, the level of IL-10 was 1,3 times lower

Table

Changes in TNF-α, IL-1β, IL-10, MMP-9, and IMMP-1 levels in the blood of women, as well as DNA methyltransferase 1 and 5-methyl-2'-deoxycytidine levels in leukocytes in the groups examined

Parameters studied	Group	Pre-pregnancy	6 weeks pregnant	12 weeks pregnant
IL-TNF pg/ml	1	7,1±0,9	10,8±1,3*	12,9±1,4 *
	2	12,7±1,3^{o}	17,9±1,8*^{o}	23,7±2,5*^{o}
	3	28,3±2,9^{o+}	59,2±6,1*$^{o+}$	84,9±8,6*$^{o+}$
IL-1β pg/ml	1	4,2±0,5	8,3 ± 0,9*	10,1±1,2*
	2	8,9±0,9 ^{o}	14,8 ± 1,5*^{o}	19,7±2,1*^{o}
	3	22,4±2,3 $^{o+}$	43,7 ± 4,4*$^{o+}$	62,8±6,4*$^{o+}$
IL-10 pg/ml	1	9,3±1,2	7,4±0,8	5,9±0,7*
	2	11,2±1,3	8,5±0,7	6,4±0,5*
	3	3,7±0,4 ^{o}	2,1±0,24*^{o}	1,4±0,17*^{o}
MMP ng/ml	1	0,7 ± 0,04	0,9 ± 0,07*	1,2± 0,11*
	2	1,1 ± 0,09 ^{o}	1,5 ± 0,13*^{o}	1,8± 0,16*^{o}
	3	1,7 ± 0,15 $^{o+}$	3,1 ± 0,28*$^{o+}$	4,3 ± 0,39*$^{o+}$
IMMP ng/ml	1	1,1 ± 0,07	0,8 ± 0,06*	0,6± 0,05*
	2	1,3 ± 0,10	1,1 ± 0,09 ^{o}	0,8± 0,07*^{o}
	3	0,9 ± 0,08	0,6 ± 0,04*$^{o+}$	0,3 ± 0,01*$^{o+}$
DNA methyltransferase 1 nM/m	1	38,6±3,5	27,1±2,4*	21,3±1,9*
	2	46,8±4,3	34,2±3,2*	28,4±2,5*
	3	61,2±5,8 ^{o}	74,6±7,1 $^{o+}$	83,5±7,9*$^{o+}$
5-methyl-2'-deoxycytidine ng/ml	1	81±7,7	59±5,4*	48±4,5*
	2	96±9,2	72±6,8	61±5,7*
	3	133±11,7 $^{o+}$	148±13,5 $^{o+}$	162±14,9 $^{o+}$

Note: 1 - women with a full-term pregnancy and no urogenital infections; 1 - women with a full-term pregnancy and urogenital infections; 2 - women with miscarriages and urogenital infections.

* - significantly different values from pre-pregnancy values.

^{o} - significantly different values from Group 1 values.

$^{+}$ - significantly different values from Group 2 values.

than at 6 weeks, but at the same time it was 1,8 times higher than the values before pregnancy, remaining at a level close to the values of women from the first group (the difference was also not statistically significant). In the third group, the level of IL-10 before pregnancy was significantly lower than in the first and second groups. Specifically, it was 2,5 times lower compared to the values of the first group and 3,0 times lower compared to the results of the second group. At 6 weeks of pregnancy, IL-10 levels in the third group were 1,8 times lower than before pregnancy and significantly lower than those in the first and second groups (3,5 and 4,0 times, respectively). At 12 weeks of pregnancy, IL-10 levels in the third group remained lower, with a 2.6-fold difference compared to pre-pregnancy levels, a 4,2-fold difference compared to the first group, and 4,6 times lower than those in the second group.

The studies showed that in the second group, MMP blood levels before pregnancy were significantly higher (1,6 times) than in the first group. At 6 weeks of pregnancy, this indicator in this group increased by 1,4 times compared to pre-pregnancy values and was 1,7 times higher than in the first group. At 12 weeks of pregnancy, the MMP level in Group 2 was slightly lower than at 6 weeks (by 1,2 times), but was significantly higher than before pregnancy (by 1,6 times) and 1,5 times higher than in Group 1. In Group 3, the MMP level before pregnancy was significantly higher than in Group 1 (by 2,4 times) and 1,6 times higher than in Group 2. At 6 weeks of pregnancy, this indicator in Group 3 increased by 1,8 times compared to baseline data and was also 3,4 times higher than in Group 1 and 2,1 times higher than in Group 2. At 12 weeks of pregnancy, the MMP level in Group 3 was significantly 2,5 times higher than pre-pregnancy values, 3,6 times higher than in Group 1, and 2,4 times higher than in Group 2.

Analysis of MMP data in Group 2 before pregnancy revealed a slight (non-significant) increase of 1,2 times compared to Group 1. At 6 weeks of pregnancy, the same women's MMP levels were statistically significantly higher than Group 1 values by 1.4 times, but showed a non-significant decrease of 1,2 times compared to their pre-pregnancy data. By the 12th week of pregnancy, a reliable decrease in the indicator was noted in group 2 by 1,6 times lower than the initial level (before pregnancy), 1,4 times lower than the values of the 6th week and 1,3 times higher than in group 1. In women in group 3, the level of IMMP before pregnancy was insignificantly lower by 1,2 times compared to group 1 and 1,4 times lower relative to group 2. At the 6th week of pregnancy, a statistically significant decrease in the indicator was recorded in this group by 1,5 times lower than the initial values, 1,3 times lower than group 1 and 1,8 times lower than group 2. By the 12th week of pregnancy, in group 3 the level of IMMP reached minimum values, demonstrating a reliable decrease of 3,0 times relative to pre-pregnancy data, 2,0 times lower than the indicators of group 1 and 2,7 times lower than group 2.

The study results revealed significant differences in DNA methyltransferase 1 (DNMT1) activity between the groups before pregnancy and at different gestational periods. In Group 2, DNMT1 activity was

slightly 1,2 times higher before pregnancy than in Group 1. At 6 weeks of pregnancy, a significant decrease in the indicator relative to the pre-pregnancy result by 1,4 times was noted, while the values remained 1,3 times higher than in Group 1. By 12 weeks of pregnancy, DNMT1 activity decreased by 1,2 times compared to week 6, demonstrating a significant difference from the pre-pregnancy level of 1,7 times and an excess of Group 1 values by 1,3 times. In Group 3, DNMT1 activity before pregnancy was significantly higher than in Group 1 by 1,6 times and Group 2 by 1.3 times. At 6 weeks of pregnancy, a slight increase in activity relative to pre-pregnancy levels was noted (1,2 times), with values significantly exceeding those in Group 1 (2,8 times) and Group 2 (2,2 times). By 12 weeks of pregnancy, DNMT1 activity was 1.4 times higher than pre-pregnancy levels, reaching maximum differences of 3,9 times in Group 1 and 2,9 times in Group 2.

The results of the studies showed that the level of 5-methyl-2'-deoxycytidine in the blood of women in the second group before pregnancy was only slightly higher than in women in the first group, by 1,2 times. At the 6th week of pregnancy, this indicator in women in the second group was slightly lower, amounting to insignificantly 1,3 times less than before pregnancy, but at the same time remained insignificantly 1,2 times higher than in women in the first group. At the 12th week of pregnancy, the level of 5-methyl-2'-deoxycytidine in group 2 was slightly lower compared to the 6th week by 1,2 times, but remained 1,6 times higher than before pregnancy, and 1,3 times higher than the indicators of the first group. In women in the third group, the level of 5-methyl-2'-deoxycytidine before pregnancy was significantly higher than in women in the first group (by 1,6 times) and the second group (by 1,4 times). At 6 weeks of pregnancy, this indicator was slightly higher than baseline values, 1,1 times higher, 2,5 times higher than in women in Group 1, and significantly higher than in Group 2, 2,1 times higher. At 12 weeks of pregnancy, 5-methyl-2'-deoxycytidine levels in Group 3 showed minor changes, remaining 1,2 times higher than pre-pregnancy levels, significantly higher than Group 1, 3,4 times higher, and 2,7 times higher than Group 2.

The study results revealed significant differences in the women's immune-inflammatory and epigenetic profiles in early pregnancy, which is important for assessing the risk of complications. In Group 2, levels of proinflammatory interleukins (TNF-α, IL-1β) were higher before pregnancy compared to Group 1 and continued to increase until 12 weeks of pregnancy. At the same time, IL-10 levels in this group were slightly higher than in the first group, and by the 12th week of pregnancy, they had stabilized or slightly decreased compared to pre-pregnancy levels. These changes, judging by the dynamics of the ratio of pro-inflammatory and anti-inflammatory interleukins, are within the normal regulatory range, indicating a balanced immune response and the absence of a tendency toward increased pro-inflammatory processes. Thus, the body effectively copes with infectious agents, creating optimal conditions for maintaining pregnancy.

Regarding matrix metalloproteinases (MMPs), women in the second group had higher levels, which continued to increase until the 12th week of pregnancy. At the same time, tissue inhibitor of matrix metalloproteinases (TIMPs) levels in the second group were higher before pregnancy than in the first group, and although they decreased at weeks 6 and 12, they remained higher than those in the first group. Increased MMP levels and decreased IMMP levels may contribute to increased proinflammatory responses, and an imbalance in the MMP/IMMP ratio during severe infections may lead to uncontrolled proteolytic activity and increased inflammation. The data obtained confirm that changes in both MMP and IMMP levels within normal limits indicate a balanced ratio of these parameters, which, in turn, contributes to a favorable pregnancy course, despite the presence of infection.

A study of DNA methyltransferase 1 activity in leukocytes in the second group revealed a slight decrease in its activity compared to pre-pregnancy levels. However, this indicator remained slightly higher than in the first group. Similar changes were also recorded for 5-methyl-2'-deoxycytidine levels, which decreased compared to pre-pregnancy levels but remained slightly higher than in the first group. These results also confirm the insignificant impact of existing infections on DNA methyltransferase 1 activity and changes in DNA methylation in leukocytes, which contributes to the maintenance of stable epigenetic processes in the body and a favorable pregnancy course. The results of the third group significantly exceeded those of the second and first groups. For example, the level of TNF-α in this group was significantly higher than in the first and second groups both before pregnancy and at 6 and 12 weeks. This may indicate increased inflammatory activity in women in this group, which is important for further clinical observations, as high levels of TNF-α are associated with the risk of inflammation and complications during pregnancy. Similar changes were observed for IL-1β, which was also significantly elevated in the third group compared to the first. IL-1β plays an important role in inflammatory processes and may be a marker of impaired immune regulation, which may subsequently be associated with an increased risk of miscarriage or other complications. Levels of IL-10, an anti-inflammatory cytokine, also showed significant differences. In Group 3, IL-10 levels were significantly lower, which may indicate a disrupted immune response, a reduced ability to suppress inflammation, and possibly an increased risk of inflammatory diseases.

Significantly elevated MMP levels in Group 3 indicate possible degradation of tissue matrix components, which may be associated with disruptions in normal uterine function, for example, during embryo implantation or the inflammatory response. Changes in TIMP levels indicate that women in Group 3, despite initially lower levels, ultimately experience more pronounced changes toward a decrease in levels, which may indicate compensatory mechanisms aimed at regulating MMPs.

Changes in DNMT1 activity in leukocytes showed that in Group 3, its activity was significantly higher than in Groups 1 and 2, which may indicate increased activity of DNA methylation processes involved in the control of gene expression, which in turn may influence

the body's adaptation to pregnancy. Changes in 5-methyl-2'-deoxycytidine levels, a DNA methylation marker, also support the hypothesis that group 3 had more pronounced epigenetic changes, possibly related to immune and reproductive function. These results suggest that an imbalance between proinflammatory and anti-inflammatory cytokines, dysregulation of MMP/IMMP, and epigenetic changes associated with DNA methylation may play an important role in the pathogenesis of pregnancy complications, especially in group 3.

Conclusions: In women with a full-term pregnancy and full-term delivery who had genital infections before pregnancy, a balanced ratio of proinflammatory (TNF-α, IL-1β) and anti-inflammatory (IL-10) cytokines, as well as balanced MMP/IMMP levels and moderate epigenetic changes, contribute to effective immune regulation, which contributes to a favorable pregnancy even in the presence of infection.

In women who had miscarriages at 12 weeks of gestation and also had genital infections before pregnancy, a significant cytokine imbalance (high TNF-α, IL-1β; low IL-10) indicates excessive inflammation and the risk of complications associated with miscarriages. A significant increase in MMPs and a decrease in TIMPs can disrupt tissue integrity, worsening conditions for pregnancy. Increased DNA methylation (elevated DNMT1 and 5-methyl-2'-deoxycytidine) indicates epigenetic abnormalities that potentially impact immune adaptation and reproductive function.

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