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Alterations in DNA Methylation of Leukocytes in Women Experiencing Early Pregnancy Loss and An Overactive Proinflammatory Immune Response

Mukhitdinova Kamola Oybekovna[1], Aleynik Vladimir Alekseevich[2], Nazhmutdinova Dilbar Kamaritdinovna[3]

[1]Assistant, PhD of the Department of Obstetrics and Gynecology, Andijan State Medical Institute, Andijan, Republic of Uzbekistan.

Email ID: kamosha.muhitdinova@mail.ru, ORCID: 0000-0003-1322-4292

[2]Doctor of Medical Sciences, Professor, Director of the Andijan branch of the Institute of Human Immunology and Genetics of the Academy of Sciences of the Republic of Uzbekistan, Andijan, Republic of Uzbekistan.

kamosha.muhitdinova@mail.ru, ORCID:007-0741-7804-4687

[3]Doctor of Medical Sciences, Professor, Head of the Department of Obstetrics and Gynecology of the Tashkent Academy of Medicine, Tashkent Medical Academy, Tashkent, Republic of Uzbekistan.

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ABSTRACT

The research examined alterations in DNA methylation patterns of leukocytes among women who experienced early pregnancy loss and displayed a significant pro-inflammatory immune reaction. The findings suggested that an intensified pro-inflammatory response during the initial phases of pregnancy, when genital infections are absent and the corrective effects of protease inhibitors are inadequate, could lead to detrimental conditions for both the progression of early pregnancy and the likelihood of miscarriages. Furthermore, adverse pregnancy outcomes may be associated with changes in global DNA methylation, manifested by a significant increase in the activity of DNA methyltransferase 1 and the index of 5-methyl-2'-deoxycytidine in leukocytes both before pregnancy and at 6 and 12 weeks of gestation. Thus, the analysis of pro-inflammatory cytokines (TNF-α and IL-1β), the anti-inflammatory cytokine IL-10, matrix metalloproteinase-9 and its inhibitor MMP-1 in blood samples, coupled with the assessment of DNA methyltransferase 1 activity and the levels of 5-methyl-2'-deoxycytidine in leukocytes, could serve as potential biomarkers for adverse outcomes in early pregnancy.

Keywords: *interleukins, protease inhibitors, DNA methyltransferase 1, early pregnancy, miscarriage, epigenetic DNA methylation*

1. INTRODUCTION

Epigenetics investigates how observable traits can be influenced by chromosomal changes that do not involve direct alterations to the DNA sequence [1]. These modifications typically occur through post-translational modifications to histone tails, such as methylation, acetylation, ubiquitination, and phosphorylation. Moreover, they are regulated by changes in transcription factors and the activity of non-coding RNAs, which contribute to transcriptional control. CpG sites often congregate in specific regions known as CpG islands (CPIs), which are commonly located at the 5' end of genes, near promoters, initial exons, or transcription start sites. The methylation of gene promoters at their 5' regions induces modifications to chromatin structure, influencing the accessibility of the transcriptional machinery required for gene expression [6].

Research has indicated that alterations in DNA methylation, specifically hypomethylation, were observed in the leukemic cells of mothers at the onset of pregnancy, in contrast to cells from non-pregnant individuals. Given that each chromosome contains distinct methylated genes, these findings imply a genome-wide hypomethylation effect. Collectively, these observations suggest that modifications in methylation patterns of leukemic DNA may serve as a temporary epigenetic strategy facilitating maternal immune tolerance and adaptation during pregnancy. In addition, the results provide preliminary evidence associating early pregnancy with alterations in the DNA methylation of maternal leukemic cells. This is primarily reflected as hypomethylation across genes on various chromosomes when compared to non-pregnant conditions [9].

Studies have shown that the methylation profile of the DNA genome of maternal leukemic cells at birth has higher methylation levels in pre-eclampsia compared to normotensive controls. The presence of methyl groups at key CpG sites alters the structure of the DNA molecule and complicates gene transcription. Increased methylation in promoter regions of maternal DNA in pre-eclampsia supports the hypothesis that many genes may be disabled or repressed compared to normal pregnancy. Previous studies have defined normal pregnancy as a state of reduced methylation compared to the absence of pregnancy. The relative hypermethylation observed in this study may in fact be a failure of hypermethylation associated with normal maternal adaptation to pregnancy [5, 8].

Some genes have been shown to be expressed in leukemic cells, but most are mainly expressed in other tissues [4]. Blood cells interact with all tissues in the body, including leukemic migration through the blood-brain barrier. Peripheral changes in the leukemic epigenome may be part of the pathophysiology of a disease or reflect epigenetic changes in distant tissues [3, 2]. A growing body of literature indicates that the presence of changes in the epigenome or transcriptome of peripheral blood can serve as a biomarker of non-hematological diseases [2]. Given the unavailability of various tissues for analysis in vivo, the concept of using a marker of expressed genes from peripheral blood for other diseases or methods of their treatment has been widely studied [10].

Purpose of the study: To explore the alterations in the DNA methylation of leukocytes associated with repeated early pregnancy loss, characterized by an inflammatory immune response and the absence of sexually transmitted infections.

2. MATERIAL AND METHODS OF RESEARCH

For this research, a total of 44 females were enlisted as participants and separated into two distinct groups. The first group consisted of 26 healthy women and the second group consisted of 18 women without genital infections and with a history of habitual abortion.

All participants provided blood samples prior to conception, as well as at 6 and 12 weeks post-conception. To assess levels of anti-inflammatory cytokines, enzyme-linked immunoassay (ELISA) was employed to quantify interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α). Additionally, interleukin-10 (IL-10), which is another cytokine with anti-inflammatory properties, was assessed using the testing system from Vectorbest in Russia. Moreover, a study was carried out on protease inhibitors, including matrix metalloproteinases (MMP-9) and the inhibitor of matrix metalloproteinase (TIMP1), employing the assay system developed by German DRG.

Assuming that methylation results in the formation of a 5-methyl-2'-deoxycytidine methylated base at the C5 position of the 2'-deoxycytidine molecule, changes in DNA methylation levels were measured using concentrations of 5-methyl-2'-deoxycytidine. Therefore, the level of 5-methyl-2'-deoxycytidine DNA methylation in the supernatant of hemocytosed leukemic cells (isolated using fecol verografin) was determined by ELISA (standard set by BCM Diagnostics, USA) at 5-methyl-2'-deoxycytidine concentration. Using the ELISA kit (standard human kit, Germany) for the determination of cytosine-5-methyltransferase-1, the activity of DNA methyltransferase 1 (DNMT1) was evaluated in the supernatant of washed hemolysed lymphocytes (isolated using fecol verografin).

3. THE RESULTS OBTAINED AND THEIR DISCUSSION

The results showed that the pre-pregnancy blood TNF-α level of subjects in group 1 (6.2 ± 0.8) was significantly lower than the level at 6 weeks (9.7 ± 1.2), while at 12 weeks the level of this indicator was higher than the level at 6 weeks (11.9 ± 1.5) (Table).

In the second cohort studied, the levels of pre-pregnancy TNF-α were markedly greater, measuring 2.3 times more than those in the first cohort (14.8 ± 1.6). When evaluating the TNF-α levels at 6 weeks of gestation in group 2, they were found to be 2.6 times higher (25.5 ± 2.7) than those in group 1, and also significantly elevated compared to the pre-pregnancy levels (6.2 ± 0.8) within the same group. By 12 weeks of gestation, the TNF-α concentrations in women from group 2 were over 2.8 times higher (32.8 ± 4.1) than those in group 1 (11.9 ± 1.5), and 2.2 times greater when compared to their pre-pregnancy levels as well (Table).

Upon examining IL-1β, alterations akin to those seen with TNF-α were identified. Notably, these alterations were substantially more pronounced in women from group 2 compared to those in group 1, particularly when both groups were assessed during the early stages of pregnancy.

In women from group 1, the levels of IL-10 in the blood remained unchanged before pregnancy, measuring 9.3 ± 1.2, and did not show a decrease at the 6-week mark (7.4 ± 0.8). However, by the 12-week point, these levels were notably reduced to 5.9 ± 0.7, signifying a significant decline compared to the levels observed at 6 weeks.

Table.: Changes in TNF-α, IL-1β, IL-10, MMP-9, TIMP1 in the blood of women, as well as DNA methyl transferase 1 and 5-methyl-2'-deoxycytidine in leukocytes of the examined groups

	The studied indicators	Group	12 weeks of pregnancy	6 weeks of pregnancy	Before pregnancy
pg/ml	TNF-α	1	11,9±1,5 *	9,7±1,2*	6,2±0,8
		2	32,8±4,1 * º	25,5±2,7* º	14,8±1,6º
	IL-1β	1	10,1±1,2*	7,2 ± 0,9*	3,7±0,4
		2	25,9±3,1* º	19,4 ± 2,3* º	11,6±1,5 º
	IL-10	1	5,9±0,7*	7,4±0,8	9,3±1,2
		2	2,6±0,4* º	3,5±0,5* º	6,1±0,7 º
nm/ml	MMP-9	1	1,2± 0,11*	0,9 ± 0,07*	0,7 ± 0,04
		2	1,6 ± 0,13* º	1,3 ± 0,11* º	1,0 ± 0,08 º
	TIMP1	1	0,6± 0,05*	0,8 ± 0,06*	1,1 ± 0,07
		2	0,3 ± 0,02* º	0,5 ± 0,04* º	0,8 ± 0,06 º
	5-methyl-2'-deoxycytidine	1	48±4,5*	59±5,4*	81±7,7
		2	151±13,9* º	136±12,7 º	112±9,8 º
ng/ml	DNA methyl transferase 1	1	21,3±1,9*	27,1±2,4*	38,6±3,5
		2	71,6±6,7* º	63,4±5,8 º	52,3±5,1 º

In the analysis of the second group, pre-pregnancy IL-10 levels were significantly lower (6.1 ± 0.7) compared to the first group (9.3 ± 1.2), 1.6-fold lower compared to week 6 (7.4 ± 0.8) and 2.2-fold lower compared to week 12 (2.6 ± 0.4) of pregnancy in the same group. Tellingly, the level of IL-10 in the second group at the onset of pregnancy was 2.1 times lower (6.1±0.7) than in the first group at week 6 (7.4±0.8), and 2.3 times lower at week 12 (5.9±0.7) (table).

Analysis of MMP-9 levels at 6 weeks' gestation in women in group 1 showed increased levels (1.2 ± 0.11) compared to pre-pregnancy (0.7 ± 0.04). In women in the same group, MMP-9 levels at 12 weeks' gestation were significantly higher (1.2 ± 0.11) than at 6 weeks' gestation (0.9 ± 0.07) and higher than before pregnancy (0.7 ± 0.04). The pre-pregnancy MMP-9 index was significantly higher in women in group 2 (1.0 ± 0.08) compared to women in group 1 (0.7 ± 0.04). At 6 weeks' gestation, the same index was 1.4-fold higher in women in group 2 (1.3 ± 0.11) compared to women in group 1 (0.9 ± 0.07), but significantly lower compared to pre-pregnancy (1.0 ± 0.08). MMP-9 levels were 1.3-fold higher in women in group 2 (1.6 ± 0.13) compared to women in group 1 (1.2 ± 0.11) and 1.6-fold higher in women in the same group compared to pre-pregnancy (1.0 ± 0.08) at 12 weeks' gestation (Table).

Analysis of the TIMP1 index in women in group 1 showed that the value at 6 weeks of gestation (0.8 ± 0.06) was significantly lower than before pregnancy (1.1 ± 0.07). Moreover, in the same group, TIMP values at 12 weeks were significantly lower than at 6 weeks of gestation (0.6 ± 0.05) but higher than before pregnancy (see table).

On the other hand, TIMP values were significantly lower in women in group 2 (1.8 ± 0.06) compared to women in group 1 (1.1 ± 0.07), and this value was 1.4 times lower in women in group 1 (0.8 ± 0.06) compared to pre-pregnancy. In women belonging to group 2, the frequency of TIMP1 was observed to be 1.6 times less (0.5 ± 0.04) compared to those in group 1 (0.8 ± 0.06). Additionally, women in group 2 exhibited a 2.7-fold reduction in TIMP1 frequency (1.1 ± 0.07) compared to women in group 1 prior to pregnancy at six weeks of gestation.

The levels of DNA methyltransferase 1 were notably elevated in group 2 women (52.3 ± 5.1) compared to those in group 1 (38.6 ± 3.5), with an increase of 1.4 times observed prior to pregnancy. At 6 weeks' gestation, DNA methyltransferase 1 in group 2 (63.4 ± 5.8) was 1.2-fold higher than before pregnancy (52.3 ± 5.1) and not particularly different, but 2.3-fold higher than in women in group 1 (27.1 ± 2.4). Moreover, at 12 weeks' gestation this indicator was 1.4 times higher (71.6 ± 6.7) than the same indicator in group 1 (21.3 ± 1.9) and 3.4 times higher than the pre-pregnancy indicator in the same group (38.6 ±

3.5) (Table).

The results of the 5-methyl-2'-deoxycytidine study show a significant decrease in women in group 1 at 6 weeks (59 ± 5.4) and 12 weeks (21.3 ± 1.9) of pregnancy. For 5-methyl-2'-deoxycytidine, it further showed a significant decrease in women in group 1 at 6 weeks (59 ± 5.4) and at 12 weeks (21.3 ± 1.9). Interestingly, the opposite dynamics was observed in women in group 2. That is, a relatively small increase at week 6 (136 ± 12.7) and a significant increase at week 12 (151 ± 13.9) compared to the same group of women before pregnancy (81 ± 7.7). Compared with similar values in the first group, a significant increase was observed at week 12 (table).

The analysis of the results of the study gives the right to conclude that a favorable inflammatory process is necessary for the onset of pregnancy. Nevertheless, an enhanced anti-inflammatory immune response in early pregnancy, even in the absence of urogenital infections and insufficient exposure to protease inhibitors, can lead to an unfavorable course of pregnancy and contribute to miscarriage. In addition, changes in epigenetic global DNA methylation can affect the unfavorable course of pregnancy, as evidenced by a significant increase in the activity of DNA methyltransferase-1 and 5-methyl-2'-deoxycytidine in leukocytes both before conception, in the first trimester, and in particular at the 6th and 12th weeks of pregnancy. The levels of inflammatory interleukins such as TNF-α and IL-1β, together with the anti-inflammatory interleukin IL-10, may signify adverse pregnancy outcomes in the first trimester. Additionally, the presence of matrix metalloproteinase-9, its inhibitor matrix metalloproteinase-1, and the activity of DNA methyltransferase-1, along with the measurement of 5-methyl-2'-deoxycytidine related to leukocyte counts in the blood, can also serve as indicators of deteriorating pregnancy conditions. These biomarkers are critical for assessing the potential risks associated with early pregnancy developments. By monitoring these specific proteins and enzymes, healthcare professionals can gain insights into the inflammatory processes occurring in the body, allowing for early intervention if necessary. Tracking these levels provides valuable information on the mother's condition and the potential for complications, thereby supporting better management of pregnancy outcomes during these crucial initial weeks.

4. CONCLUSION

The results of this study suggest that even in the absence of sexually transmitted infections and minimal exposure to protease inhibitors during the initial phases of pregnancy, an intensified anti-inflammatory immune response can lead to adverse outcomes, notably miscarriage. Furthermore, the unfavorable progression of pregnancy may be affected by epigenetic alterations in global DNA methylation. This is evidenced by a notable rise in the activity of DNA methyltransferase-1 as well as 5-methyl-2'-deoxycytidine levels in leukocytes, observed both prior to conception and during the early weeks of gestation, specifically at the 6-week and 12-week marks. Consequently, the concentrations of pro-inflammatory interleukins (TNF-α, IL-1β) and the anti-inflammatory interleukin (IL-10), along with the levels of matrix metalloproteinase-9 and matrix metalloproteinase-1 inhibitors in the bloodstream, as well as the activity of DNA methyltransferase-1 and the presence of 5-methyl-2' deoxycytidine in leukocytes, indicate an adverse pregnancy trajectory during the initial stages. These biomarkers reflect the inflammatory state of the body and can provide insights into the health of the pregnancy. Monitoring these substances helps identify potential complications early on, allowing for timely interventions if necessary. The interplay between these inflammatory mediators and DNA methylation further underscores the complexity of the biological processes at play in early gestation, highlighting the importance of regulating these factors to promote a favorable outcome. Understanding these elements is essential for healthcare professionals who aim to support women during pregnancy and address any emerging risks effectively.

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