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INFLAMMATION AS A RISK FACTOR OF PRETERM DELIVERY

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Spontaneous preterm delivery remains the leading cause of neonatal mortality and morbidity, especially in gestational periods less than 28 weeks. The content and ratio of CD-4 and CD-8 lymphocytes, as well as the concentration of interleukin-1 and interleukin-10 in the blood serum, were studied in 90 women with premature labour at 24–28 weeks and in 91 women with preterm delivery at 28–34 weeks. The control group consisted of 32 pregnant women with an uncomplicated course of pregnancy. It has been shown that in women with preterm labour at 28–34 weeks, the number of T-helpers and the concentration of pro-inflammatory cytokines was significantly higher than in healthy pregnant women. The CD-4/CD-8 index in both groups of preterm delivery was high at 1.2, while in healthy pregnant women, it was 0.8. The identified differences justify the development of treatment tactics in different gestational terms.

Key words: preterm delivery, interleukin-1, interleukin-10, T-helpers, T-suppressors.

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ЗАПАЛЕННЯ ЯК ФАКТОР ПЕРЕДЧАСНОЇ ПОЛОГОВОЇ ДІЯЛЬНОСТІ

Спонтанне передчасне розродження залишається провідною причиною неонатальної смертності та захворюваності, особливо в гестаційні терміни менше 28 тижнів. У 90 жінок з передчасної пологової діяльністю в терміни 24–28 тижнів, а також у 91 жінки з передчасними пологами в терміни 28–34 тижні вивчено зміст і співвідношення CD-4 і CD-8 лімфоцитів, а також концентрацію інтерлейкіну-1 та інтерлейкіну-10 в сироватці крові. Контрольну групу створили 32 вагітні з неускладненим перебігом вагітності. Показано, що у жінок з передчасної пологової діяльністю в терміни 28-34 тижнів кількість Т-хелперів і концентрація прозапальних цитокінів значно більше, ніж у здорових вагітних. Індекс CD-4\CD-8 в обох групах передчасної пологової діяльності був великим 1,2, в той час, як у здорових вагітних – 0,8. Виявлені відмінності обґрунтовують розробку тактик лікування в різні гестаційні терміни.

Ключові слова: передчасні пологи, інтерлейкін-1, інтерлейкін-10, Т-хелпери, Т-супресори.

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The standard implementation of all human body functions, including reproductive ones, is based on a balance of biologically active substances with an antagonistic effect. The interaction of insulin and counterregulatory factors, thrombosis and fibrinolysis factors, pro- and anti-inflammatory cellular messengers are examples of such consistency. The latter ones affect all human body processes, having a paracrine and autocrine principle of action, pleiotropic effects. Pregnancy as a particular period in a woman's life is no exception, and the leading role of cytokines in its ordinary and pathological course is beyond doubt. Normal implantation is accompanied by a complex immune response based on inflammation: the presence of specific antigens on the surface of the formed trophoblast that stimulate the formation of incomplete (blocking) antibodies. The importance of these antibodies is to bind trophoblast antigens, which protects them from attacks by active peripheral blood lymphocytes. This process is impossible without activating the pro-inflammatory part of the immune system [6]. Pro-inflammatory IL-8 with pronounced local activity contributes to synchronous changes in the cervix before ovulation: its softening and increased cervical mucus secretion [8]. However, the further course of pregnancy requires a stable dominance of the anti-inflammatory part of the immune system [7]. Thus, a long-term study of women with normal pregnancy and recurrent miscarriage showed that the optimal ratio of T-helpers and T-suppressors is less than 1.03 [5]. Immune imbalances can lead to various pregnancy complications, including preeclampsia, placental dysfunction, and more. However, our attention was focused on preterm delivery and the possible role of the inflammatory process in its origin. Preterm delivery (PD), labour before the gestational age of 37 weeks – complicates from 3 to 10 % of all pregnancies, creating the leading cause of neonatal mortality and morbidity. The search for etiological factors and pathogenetic mechanisms of preterm labour has led to the formulation of numerous theories and preventive options. Still, their implementation has not led to a decrease in the prevalence of this pathology.

The action of anti-inflammatory mediators on the uterus is realised by activating the arachidonic cascade, the end products of which–prostaglandins – are the leading factor in the contractile activity of the uterus. The cervix is also very sensitive to proinflammatory IL action. Here, they cause the activation of various origins of collagenases, which leads to increased tissue hydrophilicity, elasticity, and reduced

ability to perform occlusal function [5]. Classical views about the infectious process as a leading risk factor for PD are also based on the dominance of the pro-inflammatory link of immune homeostasis. After all, neither the specific localisation, nor the specific pathogen, nor the critical count of microorganisms that would initiate premature labour have been proven. In the presence of acute or chronic infectious processes, the risk of PD depends on the initial level of activity of both pro- and anti-inflammatory parts of the immune system and mainly on their ratio.

Clinically, this is due to the uneven effectiveness of tocolytics: in particular, β-adrenergic agonists lose some of their activity in an acidic environment, which is formed within the inflammatory. The tocolytic effect of the nonsteroidal anti-inflammatory drug indomethacin was recognised by one meta-analysis as optimal in terms of effectiveness/cost/side effects [11]. In addition, the difference between preterm labour (22–28 weeks) and early preterm delivery (28–34 weeks) is not limited to the degree of the immaturity of the newborn, but also in the clinical course of labour and the different effectiveness of drugs designed to block it. The lack of a universal tocolytic medicine for all pregnant women with preterm labour prompted the study of immunological differences between women at different gestational periods.

The purpose of the study was to access the state of pro- and anti-inflammatory parts of the immune system in women in labour at the beginning of the first period of premature delivery at different gestational ages.

Material and methods. We examined 213 pregnant women, which were divided into 3 groups. The first and second groups were formed by women hospitalised in the Kyiv Perinatal Center during the 2018–2019 years at the beginning of the first period of preterm delivery. Criteria for inclusion were the presence of regular uterine contractions (3 in 10 minutes) and structural changes of the cervix (its shortening and dilating not less than 3 cm but not more than 4 cm, i.e., women in the latent phase of the first period of labour). Group I consisted of 90 women in labour with PD in the gestational period of 24–27 weeks, Group II – 91 pregnant women with preterm delivery at 28–34 weeks. Group III consisted of 32 pregnant women (control group) with an uncomplicated course of pregnancy with a period of 24 to 32 weeks, subject to further delivery within physiological terms. In all women, the concentration of interleukin-8 (IL-8) and interleukin-6 (IL-6) as the leading representatives of the pro-inflammatory link and interleukin-10 (IL-10), an essential anti-inflammatory mediator for pregnancy progression, was studied by enzyme-linked immunosorbent assay. Also, immunological parameters were studied in all women. Namely, the number and ratio of CD-4 and CD-8 subtypes of lymphocytes determined by the rosetting method. The substrate for CD-4 fraction determining was sheep red blood cells for CD-8 – complement. Laboratory tests were performed based on a certified laboratory of Kyiv Perinatal Center. Statistical processing of the results was performed using Fisher's angular transformation criterion (for comparison of group averages), as well as the Kolmogorov-Smirnov test (to confirm differences in the distribution of patients in groups with regular, decreased or increased cytokine content).

Results of the study and their discussion. In particular, in Group I, 69 (76.7 %) of 90 patients manifested one or more episodes of respiratory infection or exacerbation of the chronic inflammation, in Group II – 73 out of 91 patients (80.3 %), and 30 (93.8 %) out of 32 healthy women remarked an episode of an infectious nature during the pregnancy. The PD frequency in the population reaches 10 %, every fifth occurring at a gestational age of fewer than 28 weeks (preterm delivery). This category of childbirth is of the most significant practical importance for neonatologists in terms of survival and future health of the newborn, which justifies their allocation to a separate group in this study.

Women with a physiological course of pregnancy had a proinflammatory IL-8 within the average reference values (table 1). Instead, women from the preterm delivery group differed in the concentration of the studied proinflammatory mediator.

Table 1

The concentration of the studied cytokines in the examined pregnant women

Cytokine type, pg/mL	Group I (n=90)	Group II (n=91)	Group III (n=32)
IL-8	84.32±1.78*#	194.32±0.87*	42.78±0.84
IL-10	12.62±0.23#	13.42±0.28*	92.52±0.99

Note * – differences are probable compared to the control group, # – differences are possible compared to Group II.

Serum IL-8 content in women with PD occurring at 28–34 weeks was four times higher than in women with physiological pregnancy, which confirms the concept of the inflammatory mechanism of the pathogenesis of preterm labour. The wide range of reference values of biological messengers does not allow to form a threshold concentration that would allow predicting PD. In addition, IL-8 has a pronounced topical activity. According to our preliminary data, the increase in its concentration in cervical mucus is characteristic of women with cervical insufficiency and irreversible structural changes in the cervix. An

increase in this concentration is recorded during the observation, explaining the progression of shortening and smoothing the cervix.

Given the broad reference values of IL content (the average concentration for non-pregnant women is from 10 to 50 pg/mL), it was essential to analyse the distribution of patients by these indices (table 2).

Table 2

Distribution of pregnant women by serum cytokine concentrations

IL content	Group I (n=90), abs. (%)	Group II (n=91), abs. (%)	Group III (n=32), abs. (%)
IL-8			
Decreased	44 (48.9)	10 (11.0)[@]	21 (65.6)
Regular	35 (38.9)	20 (22.0)	7 (21.9)
Increased	11 (12.2)	61 (67.0)	4 (12.5)
IL-10			
Decreased	44 (48.9)[@]	32 (35.2)[@]	3 (9.4)
Regular	42 (46.7)	39 (42.9)	7 (21.9)
Increased	4 (4.4)	20 (21.9)	22 (68.7)

Notes: @ – $\lambda_{empir.}$ More than $\lambda_{crit.}$ compared to Group III.

As predicted by classical views of reproductive immunology, the group of healthy pregnant women was dominated by women with low concentrations of proinflammatory IL-8 – 65.6 %. Only 4 women in this group (12.5 %) showed increased mediator content.

Distribution analysis shows that the vast majority of patients in group II (preterm labour after 28 weeks), namely 67 %, had an increased concentration of pro-inflammatory IL-8. This significantly distinguishes them from healthy pregnant women (only 12.5 %), which corresponds to the observed increase in the average concentration in the group.

Various immunocompetent cells, both of blood and tissue origin, are the source of IL-8 formation. Unlike other cytokines, IL-8 has a limited pool of target cells, mainly neutrophils. The effect of cytokine on other cells is short-lived and insignificant. In contrast, neutrophils show active migration to the site of inflammation (actually, to the area of cytokine formation), secretion of granular enzymes, and intense connective tissue degeneration in response to IL-8 stimulation. These changes can affect the cervix, causing it to shorten and soften, and their implementation in the amniotic membranes leads to the risk of premature rupture. Increased serum IL-8 was considered a possible independent risk factor for PD in women without signs of uterine contractile activity. The growth of IL-8 is a more sensitive response than even the well-studied emergence of C-reactive protein.

In addition, the high content of IL-8 in biological fluids may predict the low success of tocolytic therapy. The mechanisms of such dependence may be related to the primary cause of the inflammatory process, which is localised in the placenta or amniotic membranes, or with the peculiarities of the most common tocolytics, which lose effectiveness in an environment with reduced acid-base balance as a result of inflammatory reactions.

Despite expectations, the pregnant women with the onset of labour before 28 weeks of gestation had a median concentration of IL-8. However, it was higher than in the control group but much lower than in patients with childbirth at 28–34 weeks.

Analysis of the distribution of patients in group I by the concentration of IL-8 in serum indicates that most of them showed a decreased value (48.9 %), which had no statistical differences from the group of healthy pregnant women. Only 12.2 % of women with preterm labour before 28 weeks had increased levels of one of the pro-inflammatory cytokines.

There was a natural increase in the concentration of anti-inflammatory IL-10 from the reference values in pregnant women of the control group. In the vast majority of women in the control group (68.7 %), the content of the mediator was higher than the reference values of the norm. In addition to maintaining immune tolerance to a semi-allogeneic fetus, anti-inflammatory mediators promote the normal functioning of the placental circulation and increase the uterine mass of the myometrium. Stretching the myometrium, in turn, stimulates the formation of anti-inflammatory cytokines. The peculiarity of IL-10 is the close connection of its expression with endothelial function, which allows combining infectious factors and endothelial dysfunction in the joint pathogenesis of premature labour.

There was a significant decrease in serum concentrations of IL-10 in all pregnant women with preterm delivery, regardless of the time of its initiation. In group II, this is easily explained by the shifted balance towards the pro-inflammatory part of the immune system, as it was dominated by women with high concentrations of IL-8. Their distribution also found that only one in five patients with preterm labour had an increase in IL-10 inherent in a normal pregnancy. In the group of women with a gestational age of

fewer than 28 weeks, this fact can be considered an independent pathogenetic factor. Only 4.4 % of the patients in this group had an increase in the content of IL-10, which is protective against pregnancy progression, and 48.9 % had a decrease in this indicator.

In women with a physiological course of pregnancy, the proportion of lymphocytes with T-helper activity (with CD-4 glycoprotein expression on the surface) was below the normal range (33–46 %), which is natural for pregnancy. The opposite pattern was found for T-suppressors (CD-8 expression). Their content is above the physiological norm, indicating the stimulation of anti-inflammatory cytokines with a protective effect on pregnancy. The CD-4/CD-8 ratio (calculated for the complete range of lymphocyte classes) was 0.8, according to the literature data.

Women with preterm labour after 28 gestational weeks showed a significant increase in the content of T-helpers, even higher than the norm in non-pregnant women, which is natural, given the high range of pro-inflammatory cytokines in group II. An insignificant decrease in the T-suppressors ratio leads to an increase in the CD-4/CD-8 index. It is worth noting that out of 91 patients in group II, only 4 had an index value less than 1. Immunologists consider it a necessary condition for the progression of pregnancy.

For the first group of patients, other indicators of the immunological parameters were found. They had a significant decrease in the number of T-suppressors against the background of the normal range of T-helpers, which leads to a statistically significant increase in the index. 10 patients out of 90 in this group had CD-4/CD-8 values less than 1.

Thus, both groups of preterm births showed an increase in the CD-4/CD-8 index – up to 1.2 in Group I and 1.3 in Group II. However, in women with preterm delivery in the premature period, this increase is due to a decrease in suppressor activity. In women with childbirth after 28 weeks, it results from T-helpers activation.

The identified immunological differences between PD groups at different gestational ages were clinically confirmed. So, premature labour in terms after 28 weeks resembles timely delivery: painful contractions, gradient and predicted dilating of the cervix. The contractile activity of the uterus is the result of the formation of a large number of eicosanoids, which increase the number of oxytocin receptors in the myometrium. On the other hand, extremely premature PD is often accompanied by more minor painful contractions and an unpredictable dilating process. These women are dominated by manifestations of deficiency of the anti-inflammatory part of the immune system, which regularly provides stability of the collagen in the cervical canal.

The scientific novelty of the proposed study is to find immunological differences between premature labour at different gestational ages, studied both at the level of cytokine balance and at the level of classical immunological parameters.

The identified patterns cannot be considered an independent etiological factor of premature labour. Cytokine imbalance in such cases was a secondary phenomenon, acting as a component of the pathogenesis of other, already proven mechanisms of abortion. In particular, the infectious process's role, which was considered the leading cause of PD until recently, receives a deeper explanation due to the results obtained. S.Rajaei et al. (2011), studying the role of cytokine balance in the endometrium in recurrent miscarriage, showed that premature contractile activity of the uterus depends not so much on the type of infectious pathogen but the local immune response. The literature has repeatedly raised the question of antibacterial drugs as adjuncts tocolytic therapy, but it has not been proven [9]. As mentioned earlier, the effectiveness of the indomethacin is explained by counteracting the excessive circulation of pro-inflammatory cytokines.

The role of anti-inflammatory cytokines in the physiological prolongation of pregnancy cannot be denied. Thus, a new concept of actively enhancing the formation of messengers with anti-inflammatory activity during pregnancy has recently been described. Alijotas-Reig et al. (2014) attribute this role to a specific subtype of T-lymphocytes expressing the Foxp3 factor. The value of these cells is the allo-regulation of the function of T-helpers. The growth of their activity has been registered since the first days of pregnancy, resulting in increased synthesis of anti-inflammatory mediators, in particular – IL-10 and transforming growth factor [1]. With this in mind, the content of anti-inflammatory cytokines in serum was also studied using the example of IL-10 to assess the inflammatory process in women with PD. This mediator is the most influential representative of anti-inflammatory interleukins. Its action is not limited to competition for receptors with pro-inflammatory factors; it can also inhibit the formation of tumour necrosis factor and the most pronounced anti-atherogenic among cytokines [10]. The source of IL-10 is immunocompetent cells and endothelial cells, and other tissues. Its primary function is to inhibit the formation of all pro-inflammatory mediators, both by direct action on cells that synthesise them and by expressing particular fractions of receptors to them, which bind cytokine for a long time but do not realise its action.

Data on anti-inflammatory deficiency as a leading mechanism of reproductive loss before the age of 28 weeks also correlate with the results of other studies. Thus, Nakagawa K. et al. (2015) showed the critical importance of the ratio of T-helpers and T-suppressors as an independent factor in the favourable course of pregnancy. Given the leading role of the endothelium in stimulating the anti-inflammatory part of the immune system, participation in its implementation of hemostasis factors, the lack of anti-inflammatory mediators in patients with highly premature labours indicates a defective restructuring of this critical system. Given the increasing attention to congenital thrombophilia as a pathogenetic factor in various obstetric complications [3], the data obtained can significantly simplify the diagnostic search and, accordingly, the choice of optimal treatment tactics. In particular, the normalisation of the function of the developing placental endothelium with cardiac doses of aspirin, which is successfully achieved in pregnant women with a proven thrombophilia, effectively prevents various obstetric complications, including PD.

Conclusions

1. Pregnant women with preterm labour at 28–34 weeks of gestation are characterised by an increased serum concentration of pro-inflammatory IL-1 and the proportion of T-helpers in the immunological parameters.

2. Pregnant women with preterm labour up to 28 weeks of gestation are characterised by reduced serum concentrations of IL-10 and the proportion of T-suppressors in the immunological parameters.

3. The practical significance of these results lies in the reasonable need to develop different approaches to the examination and treatment of women with preterm labour during gestational ages up to 28 weeks and 28–34 weeks. Thus, the dominance of the pro-inflammatory link in the period after 28 weeks involves diagnostic search and remediation in the focus of infection, then up to 28 weeks – examination to identify markers of endothelial dysfunction, in particular, thrombophilic conditions.

Prospects for further research. Further study of the immune response features in premature delivery at different gestational ages will allow developing appropriate treatment tactics to increase its efficacy.

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