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FEATURES OF IMMUNOLOGICAL BLOOD
MARKERS IN PREMATURE NEWBORNS**S. Mukhamedova¹, S. Ganieva¹,
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(Bukhara, Uzbekistan)**Summary**

Premature birth remains a global healthcare issue due to ongoing challenges in prediction and prevention. Current predictors are limited by low effectiveness, the need for invasive sampling, and the inability to identify at-risk patients in a timely manner to ensure effective intervention. Multiple etiologies of preterm birth often involve an inflammatory component. Therefore, a deeper understanding of the inflammatory mechanisms involved in preterm birth may provide opportunities for identifying new predictors of preterm labor.

Research objective: To study certain indicators of immune status in premature newborns, taking into account feeding practices.

Research materials and methods. The study involved 120 newborns, divided into 4 groups: the first control group consisted of 20 healthy newborns exclusively breastfed; the second control group included 20 healthy newborns receiving mixed feeding; the first main group consisted of 40 newborns with extremely low birth weight (ELBW) who were on parenteral nutrition with minimal amounts of expressed breast milk (EBM); the second main group included 40 newborns with low birth weight (LBW) who were on mixed enteral feeding, receiving 70% native EBM and 30% breast milk substitutes in their daily nutrition. Informed consent was obtained from the child's parents for participation in the study. Statistical processing was performed using licensed software Statistica (StatSoft Inc., Version 7) and Microsoft Excel (AtteStat, Version 12.5).

The results of the study A correlation was observed between the serum immunoglobulin G (IgG) levels and both gestational age and birth weight. In the first main group, mean IgG levels were 1.6 times lower than those of healthy exclusively breastfed newborns. In the second main group, IgG levels were 2.5 times lower compared with the first control group, which was statistically significant and indicated the morphological immaturity of the immune system, correlating with the gestational age of the newborns. Interleukin 10 (IL-10) were elevated in newborns with ELBW in the first main group (11.02 ± 1.18 pg/mL) and in the second main group with LBW (10.86 ± 1.20 pg/mL), representing a 1.4-fold increase compared with the first control group (8.30 ± 1.11 pg/mL) and a 1.7-fold increase compared with the second control group (6.25 ± 0.59 pg/mL) ($p < .05$). Cytokine profiling in premature newborns revealed elevated interleukin 1 β (IL-1 β) levels in the first group (7.92 ± 0.67 pg/mL) and in the second group (5.78 ± 0.78 pg/mL).

Conclusion. Cytokine analysis in premature newborns demonstrated increased IL-1 β levels in the main groups compared with healthy newborns. IL-10 levels were also elevated by 1.4 times compared to control values. Serum IgG concentrations in ELBW neonates were 2.5 times lower than in healthy exclusively breastfed newborns, while serum immunoglobulin A (IgA) concentrations in the second main group (LBW neonates) were twice as high as in full-term newborns from both control groups.

Keywords: Immune Status of Preterm Infants, Cytokine Status, Feeding, Immunological Indicators.

Introduction

Preterm birth, defined as delivery before 37 completed weeks of gestation, remains a leading cause of neonatal morbidity and mortality globally. Its incidence varies worldwide but remains comparatively low in Northern European countries (5%–6%). Prediction and prevention are complicated by heterogeneous etiologies; however, obstetric history and cervical length assessment have demonstrated utility in enhancing predictive accuracy [9].

Preterm birth continues to pose a significant global healthcare challenge, primarily due to ongoing limitations in effective prediction and prevention strategies. Existing predictive methods are hindered by low sensitivity, reliance on invasive sampling techniques, and failure to identify at-risk patients early enough to allow timely intervention. Multiple etiologies underlying preterm birth commonly involve an inflammatory component. Therefore, elucidating the inflammatory mechanisms contributing to preterm labor may facilitate the identification of novel biomarkers for early detection [6].

The infant gut microbiome plays a critical role in the healthy development of multiple physiological systems and is influenced by various dietary exposures. Maternal

diet during pregnancy, along with postnatal nutritional practices, significantly affects the neonatal gut microbiota. Breastfeeding promotes colonization with beneficial microbial populations, whereas formula feeding has been associated with increased microbial diversity. Additionally, the timing of solid food introduction influences gut microbiota composition. In preterm infants, the development of gut microbiota is affected by factors such as postnatal age and the extent of breast milk intake. Interventions including the administration of probiotics and prebiotics have shown promise in reducing morbidity and mortality among preterm neonates. These findings underscore the importance of continued research to assess the long-term health outcomes associated with such interventions and to develop targeted strategies for enhancing the gut microbiota in formula-fed and preterm infants [1, 3].

Preterm birth represents a significant risk factor for neonatal health, well-being, and long-term development into adulthood. Compared with full-term neonates, preterm infants exhibit increased susceptibility to various morbidities, including thermoregulatory instability, hypoglycemia, respiratory distress, apnea, jaundice, and feeding difficulties [4].

The fetal immune system is capable of initiating a local or systemic inflammatory response upon exposure to microbial pathogens or sterile stimuli (e.g., danger signals or alarmins). The term *fetal inflammatory response syndrome* (FIRS) describes a condition marked by evidence of a systemic inflammatory reaction, frequently attributed to activation of the innate immune system. FIRS is typically diagnosed through elevated levels of acute-phase reactants in umbilical cord plasma or neonatal serum, including C-reactive protein and cytokines such as interleukin 6 (IL-6). Histopathological indicators of systemic fetal inflammation include funisitis and chorionic vasculitis. FIRS was initially identified in the context of intra-amniotic infection, particularly in cases involving preterm labor with intact fetal membranes or in association with premature rupture of membranes [7].

Research objective: To study certain indicators of immune status in premature newborns, taking into account feeding practices.

Research materials and methods

The study included 120 neonates, who were divided into four groups as follows:

- First control group (n = 20): healthy full-term neonates who were exclusively breastfed.
- Second control group (n = 20): healthy full-term neonates receiving mixed feeding.
- First main group (n = 40): preterm neonates with extremely low birth weight (ELBW) who received parenteral nutrition with a minimal amount of expressed breast milk (EBM).
- Second main group (n = 40): preterm neonates with low birth weight (LBW) and either acute or chronic neonatal respiratory disease (ONRD), who received mixed enteral feeding consisting of 70% native expressed

maternal milk and 30% breast milk substitute (Pre-NAN, Nestlé, Switzerland) as part of their daily nutritional intake.

This group distribution was designed to ensure representativeness and the objectivity of comparative analysis, as the study aimed to evaluate the immunobiochemical status of neonates in relation to feeding type.

No statistically significant differences were found among the groups regarding gestational age, birth weight, initial postnatal weight loss and the time required for its recovery, the presence of adverse perinatal factors, or the administered treatments. This confirmed the homogeneity of the study cohorts. Informed consent was obtained from the child's parents for participation in the study. Statistical processing was performed using licensed software Statistica (StatSoft Inc., Version 7) and Microsoft Excel (AtteStat, Version 12.5).

The results of the study

To evaluate cytokine status, serum levels of interleukin 1β (IL-1β) and interleukin 10 (IL-10) were measured in all study groups (Table 2).

The analysis demonstrated elevated IL-1β concentrations in preterm neonates. Specifically, the IL-1β level in the first main group was 7.92 ± 0.67 pg/mL, while in the second main group it was 5.78 ± 0.78 pg/mL.

The mean birth weight in the first main group was 840.5 ± 20.3 g, which was approximately half that observed in the second main group, where the mean was 1713.7 ± 62.6 g. Among healthy full-term neonates in the first control group, the mean birth weight was 3254.2 ± 76.3 g, with a gestational age of 38.5 ± 0.8 weeks and a length of 50.7 ± 0.3 cm. In the second control group, the birth weight was 3471.2 ± 65.2 g, with a gestational age of 39.4 ± 0.7 weeks and a length of 51.6 ± 0.3 cm. In contrast, neonates in the first main group had a length of 31.8 ± 0.44 cm and a gestational age of 27.5 ± 0.41 weeks. In the second main group, the length was 39.9 ± 0.56 cm, with a gestational age of 33.6 ± 0.7 weeks (Table 1).

Table 1

Anthropometric indicators of newborns

Indicators	Control Group 1 (healthy, breastfed) n=20		Control Group 2 (healthy, mixed feeding) n=20		Group 2 (preterm infants on parenteral feeding with minimal expressed breast milk) n=40		Group 3 (preterm infants on mixed enteral feeding) n=40	
	M	m	M	m	M	m	M	m
Weight (g)	3254.2	76.3	3471.2	65.2	840.5***	20.3	1713.7**	62.6
Height (cm)	50.7	0.3	51.6	0.3	31.8***	0.44	39.9***	0.56
Gestational age (weeks)	38.5	0.8	39.4	0.7	27.5***	0.41	33.6**	0.7

Note: * – The differences compared to the control group data are significant.

(* – $P < 0.05$, ** – $P < 0.01$, *** – $P < 0.001$)

Table 2

Cytokine status of newborns

Indicators (pg/ml)	Control Group 1 (healthy, breastfed) n=20		Control Group 2 (healthy, mixed feeding) n=20		Group 2 (preterm infants on parenteral feeding with minimal expressed breast milk) n=40		Group 3 (preterm infants on mixed enteral feeding) n=40	
	M	m	M	m	M	m	M	m
IL-1β	4.54	0.65	3.48	0.43	7.92**	0.67	5.78*	0.78
IL-10	8.30	1.11	6.25	0.59	11.02**	1.18	10.86**	1.20

Note: * The values are statistically significant compared to the control group.

(* – $P < 0.05$, ** – $P < 0.01$, *** – $P < 0.001$)

In the group of extremely preterm neonates with early neonatal hypoxic encephalopathy (ENHE), the concentration of the pro-inflammatory cytokine interleukin 1 β (IL-1 β) was found to be 1.75 times higher than in

healthy neonates from the first control group (4.54 ± 0.65 pg/mL) and 2.3 times higher than in those from the second control group (3.48 ± 0.43 pg/mL) ($P < .05$) (Table 2, Figure 1).

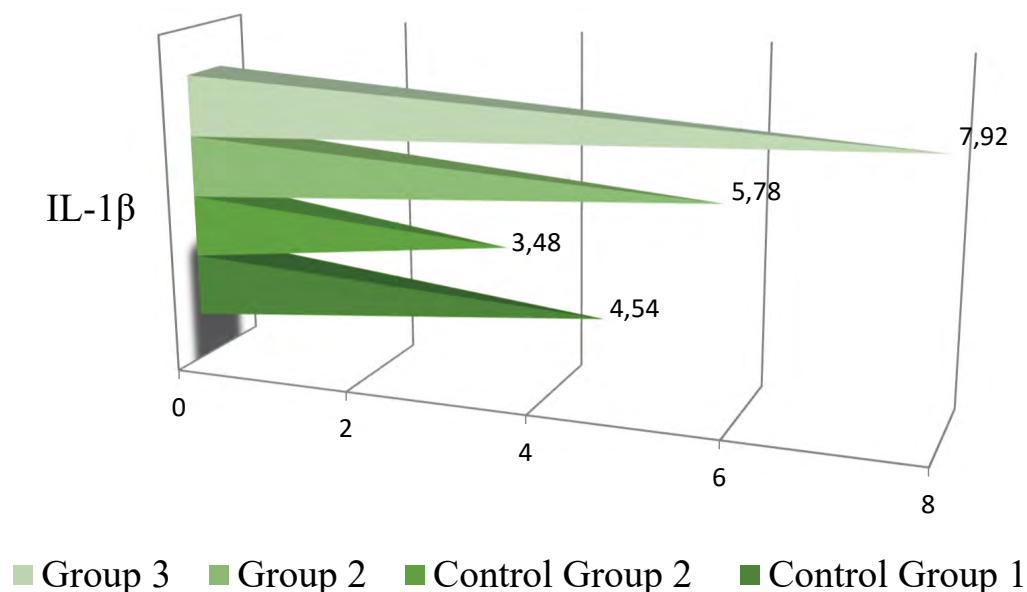


Figure 1. Cytokine IL-1 β levels in the blood of newborns.

This increase is attributed to the immaturity of the immune system associated with preterm birth and the hyperactivation of compensatory protective mechanisms. Importantly, elevated IL-1 β levels may serve as a diagnostic marker of systemic inflammatory response in this population. In our study, neonates with neonatal inflammatory diseases (NID) and elevated IL-1 β concentrations were classified as high-risk for sepsis and, consequently, received additional antibacterial therapy. The association of IL-1 β elevation with both prematurity and inflammatory pathology underscores the relevance of this cytokine in clinical risk stratification.

It is well established that the immune system plays a key role in the pathogenesis, clinical progression, and outcome of hypoxic and infectious diseases in newborns.

A critical and informative marker of immune system status during the early postnatal adaptation period is the level of cytokine production – polypeptide mediators that coordinate interactions among various physiological systems. The release of both pro-inflammatory and anti-inflammatory cytokines, along with acute-phase proteins, constitutes a nonspecific immune response of immunocompetent cells to antigenic stimuli of diverse origins. Numerous studies have emphasized the diagnostic significance of measuring serum levels of interleukin 6 (IL-6), tumor necrosis factor α (TNF- α), and interleukin 8 (IL-8) in neonates with infectious diseases, highlighting their role as reliable biomarkers of neonatal inflammatory and infectious pathology [2,8,10].

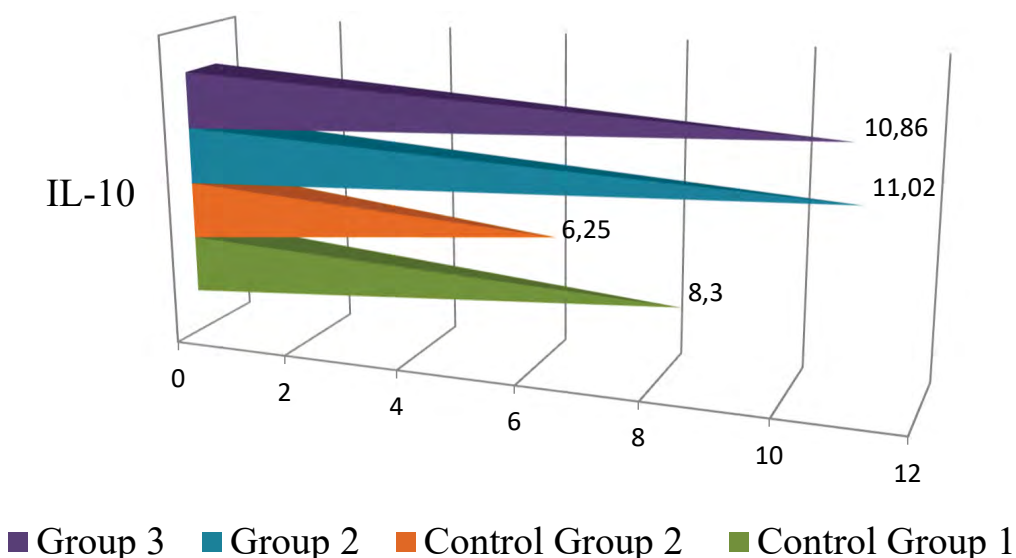


Figure 2. Cytokine IL-10 levels in the blood of newborns.

Interleukin 10 (IL-10) levels in newborns with ELBW from the first main group (11.02 ± 1.18 pg/mL) and in those with LBW and ONRD from the second main group (10.86 ± 1.20 pg/mL) were elevated by 1.4-fold compared with the first control group (8.30 ± 1.11 pg/mL) and by 1.7-fold compared with the second control group (6.25 ± 0.59 pg/mL) ($P < .05$) (Fig. 2). IL-10, as an anti-inflammatory cytokine, plays a regulatory role in pulmonary development and surfactant synthesis in the fetus [5]. Thus, elevated IL-10 levels in preterm neonates who survived episodes of respiratory distress may be considered a favorable indicator of successful adaptation to extrauterine life. In our study, the increase in IL-10 in preterm newborns was directly proportional to the stability of their condition and survival rates.

To assess the humoral arm of immunity, serum immunoglobulin (Ig) A and G concentrations in the blood of newborns were studied (Figure 4.3). A correlation was

observed between IgG levels and both gestational age and birth weight. In the first control group, the mean IgG level was 6.8 ± 0.2 g/L, compared with 5.5 ± 0.09 g/L in the second control group. In the first main group of ELBW neonates with early neonatal hypoxic encephalopathy (ENHE), the mean IgG concentration was 2.7 ± 0.2 g/L, while in the second main group of neonates with ONRD and LBW, it was 4.2 ± 0.3 g/L ($P < .01$). In the first main group, the IgG concentration was 1.6 times lower than in healthy, exclusively breastfed neonates, and in the second main group, it was 2.5 times lower than in the first control group. These statistically significant differences reflect the morphological immaturity of the immune system and correlate with the gestational age of the neonates. . Notably, in the second control group, despite functional maturity, IgG levels were 20% lower than in the first control group (Fig. 3). This variation is attributed to the use of mixed feeding practices in this group.

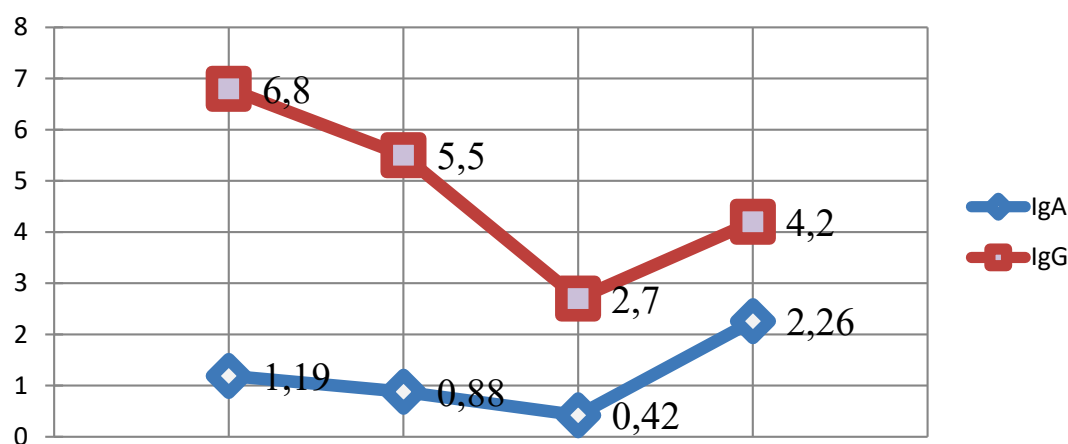


Figure 3. Levels of serum immunoglobulins A and G in newborns.

Despite the impact of exogenous and endogenous stressors on preterm neonates, premature birth and artificial feeding delay the activation of the nonspecific immune response.

Notably, interesting findings were observed regarding IgA levels. The lowest IgA concentration was recorded in the first main group, where neonates with ENHE exhibited trace levels of IgA (0.42 ± 0.15 g/L), which was 2.8-fold lower than in the first control group (1.19 ± 0.24 g/L) and twice as low as in the second control group (0.88 ± 0.19 g/L). In 18 (40%) neonates, IgA levels were below 0.1 g/L, consistent with literature data indicating that IgA synthesis at trace amounts normally begins around 30 weeks' gestation and, following antigenic stimulation by infectious agents, may start as early as the 13th to 14th week of intrauterine development. The second main group, comprising preterm neonates with ONRD and LBW, demonstrated significantly higher IgA levels (2.26 ± 0.09 g/L) compared with healthy neonates (1.19 ± 0.07 g/L). This twofold increase in IgA

among preterm neonates in the second main group relative to controls is likely attributable to the presence of a gastric probe, an invasive factor that stimulates immunoglobulin synthesis in the gastrointestinal mucosa, in combination with enteral feeding.

Conclusion. The study of cytokine status in preterm newborns demonstrated elevated IL-1 β levels in the main groups compared with healthy newborns. . IL-10 levels were also increased by 1.4-fold relative to control values.

Serum IgG concentration in newborns with ENHE was 2.5-fold lower than in healthy, exclusively breastfed infants. Conversely, serum IgA concentration in the second group of preterm newborns with acute or chronic neonatal respiratory disease (ONRD and NRD) was twice as high as in term neonates from the first and second control groups. These findings suggest distinct immune response profiles in these patient populations, potentially reflecting their underlying health conditions.

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ОСОБЛИВОСТІ ІМУНОЛОГІЧНИХ МАРКЕРІВ КРОВІ У НЕДОНОШЕНИХ НОВОНАРОДЖЕНИХ

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Резюме.

Передчасні пологи залишаються глобальною проблемою охорони здоров'я через постійні виклики у прогнозуванні та профілактиці. Існуючі предиктори обмежені низькою ефективністю, необхідністю інвазивного відбору зразків і неможливістю своєчасної ідентифікації пацієнтів для забезпечення ефективного втручання. Множинна етіологія передчасних пологів часто включає запальний компонент. Тому глибше розуміння запальних механізмів, що беруть участь у передчасних пологах, може надати можливості для виявлення нових предикторів передчасних пологів.

Матеріали та методи дослідження. У дослідженні взяли участь 120 новонароджених, які були розподілені на 4 групи: Перша контрольна група складалася з 20 здорових новонароджених, які перебували на виключно грудному вигодовуванні. Друга контрольна група включала 20 здорових новонароджених, які перебували на змішаному вигодовуванні. Перша основна група складалася з 40 новонароджених з екстремально низькою масою тіла (ЕНМТ), які перебували на парентеральному харчуванні з мінімальною кількістю зцідженного грудного молока (ЗГМ). Друга основна група включала 40 новонароджених з низькою масою тіла (НМТ), які перебували на змішаному ентеральному вигодовуванні, отримуючи в щоденному харчуванні 70% нативного зцідженного грудного молока та 30% заміника грудного молока. На участь у дослідженні отримана інформована згода батьків дитини. Статистична обробка проведена з використанням ліцензованих програм «Statistica» (StatSoft Inc., Version 7), Microsoft Excell (AtteStat, Version 12.5).

Мета дослідження: Вивчити деякі показники імунного статусу у недоношених новонароджених дітей з урахуванням вигодовування.

Результати дослідження. Спостерігалася кореляція між рівнем IgG та гестаційним віком, а також масою тіла при народженні. У першій основній групі середній рівень IgG був в 1,6 рази нижчим, ніж у здорових новонароджених, які перебували на виключно грудному вигодовуванні. У другій основній групі він був у 2,5 рази нижчим, ніж у першій контрольній групі, що було статистично значущим і свідчило про морфологічну незрілість імунної системи, яка корелювала з гестаційним віком новонародженого. IL-10 у новонароджених з екстремально низькою масою тіла (ЕНМТ) першої основної групи ($11,02 \pm 1,18$ пг/мл) та другої основної групи з малою масою тіла (ММТ) ($10,86 \pm 1,20$ пг/мл) також був підвищений в 1,4 рази порівняно з першою контрольною групою ($8,30 \pm 1,11$ пг/мл) та в 1,7 рази порівняно з другою контрольною групою ($6,25 \pm 0,59$ пг/мл) ($p < 0,05$). Дослідження цитокінового статусу у недоношених новонароджених показало підвищення рівня IL-1 β у першій групі ($7,92 \pm 0,67$ пг/мл) та в другій групі ($5,78 \pm 0,78$ пг/мл).

Висновок. Дослідження цитокінового статусу у недоношених новонароджених продемонструвало підвищення рівня IL-1 β в основних групах порівняно зі здоровими новонародженими. IL-10 також був підвищений в 1,4 рази порівняно з контрольними значеннями. Рівень IgG у сироватці крові новонароджених з екстремально низькою масою тіла (ЕНМТ) був у 2,5 рази нижчим, ніж у здорових новонароджених, які перебували на виключно грудному вигодовуванні, тоді як концентрація IgA у другій групі недоношених новонароджених з низькою масою тіла (НМТ) була вдвічі вищою, ніж у доношених новонароджених з першої та другої контрольних груп.

Ключові слова: імунний статус передчасно народжених дітей, цитокіновий статус, вигодовування, імунологічні показники.

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