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Are Lactoferrin and Interleukine-6 preterm birth participants?

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Abstract

Background: Preterm birth remains a leading obstetrical complication because of the incomplete understanding of its multifaceted etiology. It is known that immune alterations toward a proinflammatory profile are observed in women with preterm birth, but therapeutic interventions are still lacking because of scarcity of evidence in the integration of maternal and placental interrelated compartments. Objective: To investigate value of Lactoferrin (LF) and Interleukine-6 (IL-6) in the preterm labor.
Material and methods: The study comprised 65 women with spontaneous preterm labor and 65 women with term labor. Maternal plasma concentrations of Lactoferrin and Interleukine-6 were detected by standard test system Aeskulisa Lactoferrin and Best-Vector A-8768 for Interleukine-6 Ref 3307 which (GmbH & Co, Germany) gave an analytical sensitive of 1.0 U/ml for Lactoferrin and 0.131 pg/ml for Interleukine-6.
Results: Plasma levels of Lactoferrin in women with preterm labor were lower (μ_{median} = 0.90 U/ml) ($p<0.001$) than in the control subjects (μ_{median} = 40.68 U/ml). Plasma levels of Interleukine-6 in the plasma in women with preterm were predictably higher (μ_{median} = 51.90 pg/ml) than that in the control subjects with term delivery (μ_{median} = 21.51 pg/ml) ($p<0.001$).
Conclusions: The study findings suggest that plasma levels of Lactoferrin and Interleukine-6 in women with preterm labor may be considered as a promising early biomarker for preterm labor.
Key words: preterm labor, term labor, polyfunctional immunomodulatory proteins, lactoferrin.

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Introduction

Preterm birth (PTB) remains a leading cause of neonatal morbidity and mortality. PTB pertains to birth that occurs before 37 weeks of gestation, a definition that the World Health Organization has endorsed. In the European countries the rate of PTB is significantly lower (approaching 6% of all live births) than in the USA (approaching 12 % of all live births) [1-5]. Children born prematurely have an augmented risk of mortality before their fifth year of life. The financial burden of neonatal intensive care is substantial, and its emotional toll on families can last for years [1, 5].

Despite the clinical and public health significance, the etiology of PTB remains largely enigmatic. Notably, multiple causes, including environmental exposure, fetal and/or maternal genetics, stress, and immune/inflammatory conditions, have all been associated with induction of premature delivery.

Inflammation is essential throughout pregnancy – from early fetal development to labor onset [4, 5]. Inflammatory mechanisms are tightly regulated by local/systemic immune cells and mediators in uncomplicated pregnancies. However, evidence shows impaired inflammatory responses in gestational tissues and maternal circulation in pregnancy complications – notably PTB [6-10].

Although the underlying causes of pregnancy-associated complication are numerous, it is well established that infection and inflammation represent a highly significant risk factor in preterm birth. It is estimated that inflammation at the placental-maternal interface is directly responsible for or contributes to the development of 50% of all premature deliveries.

Inflammation can be induced by infections, which are detected in 20% to 30% of PTB cases [11, 12]. PTB can also occur without detectable microorganisms (sterile inflammation), whereby endogenous danger signals derived from cellular stress or necrosis, known as damage-associated molecular patterns or alarmins, are often detected [13-17].

Even more, infection and infection-driven activation of inflammatory responses are thought to be the leading risk factor of "spontaneous" PTB [9, 12, 13]. Consequently, increased production of proinflammatory cytokines has been associated with uterine activation and PTB, whereas production of anti-inflammatory cytokines has been shown to play an essential role in uterine quiescence during gestation

[2, 10]. What sets apart term and preterm labor could be an early imbalance of decidual inflammatory signals or a powerful aberrant stimulation (internal or external) that initiates inflammatory pathways. Anti-inflammatory mediators (including IL-10 and IL-4), in contrast to proinflammatory mediators (IL-1, IL-6, IL-8, TNF- and INF-), are downregulated in PTB [12, 13]. Bacterial flora in the placenta is similar to that found in the mouth rather than the vagina. Inflammation and infection have been tied to as much as one-fourth of all preterm births. The unique triple "I" approach, which represents intrauterine inflammation, infection or both, emphasizes the fact that intrauterine inflammation can manifest itself in the absence of overtly harmful intrauterine infection [18]. The findings suggest that Lactoferrine (LF) may play a role in inflammatory protection in human pregnant cervical tissue. It was clarified that LF suppresses PTB and improves the prognosis of pups in the inflammation-induced PTB animal models. Thus, we have identified the first ever clinical application of LF, a prebiotic contained in breast milk, for the purpose of suppressing PTB in humans.

In summary, a complex interplay between infection/inflammation (both systemic and i.u.) and pathogen/host biologic processes appears to play a central role in defining pregnancy outcomes. Further studies are clearly needed to better define the immune mechanisms underlying infection and/or inflammation-driven PTB [8].

The aim of the study was to investigate the levels of *Lactoferrin* and *Interleukine-6* in the maternal plasma in spontaneous preterm labor and term birth (TB).

Material and methods

During the prospective observational cohort research was performed the evaluation of the serum levels of *Lactoferrin* and *Interleukine-6* in the women who were consecutively admitted and got delivery preterm and term in the Perinatal Center of the 1st Municipal Hospital, Chișinău and gave written informed consent at the time of their admission for delivery. Birth before 37 weeks of completed gestation was considered preterm.

Maternal plasma concentrations of *Lactoferrin* and *Interleukine-6* were detected in the Biochemistry Laboratory of *Nicolae Testemitanu* State University of Medicine and Pharmacy by standard test system Aeskulisa Lactoferrin and Best-Vector A-8768 for Interleukine-6 Ref 3307 which (GmbH & Co, Germany) gave an analytical sensitive of 1.0 U/ml for *Lactoferrin* and 0.131 pg/ml for Interleukine-6.

A total of 130 women, 65 women with premature spontaneous labor were included in the 1st group; 65 women with term delivery were included in 2nd group.

The numerical values of the parameters were numbered in Excel table, after which – imported into the statistical analysis software R studio, using descriptive, variation, and correlational analysis. Applied statistic tests: non-parametric Mann-Whitney tests (with effect size determination) were used for comparative evaluation among the groups and ρ Spearman tests were applied for correlation analysis, p-values less than 0.05 being considered as threshold for statistical significance determination.

The research project was approved by the Research Ethics Committee of *Nicolae Testemitanu* State University of Medicine and Pharmacy (favorable opinion dated of 21.11.2017, No 16).

Results and discussion

A detailed analysis of the immune mediators in the maternal circulation revealed important differences in PTB in comparison with TB participants.

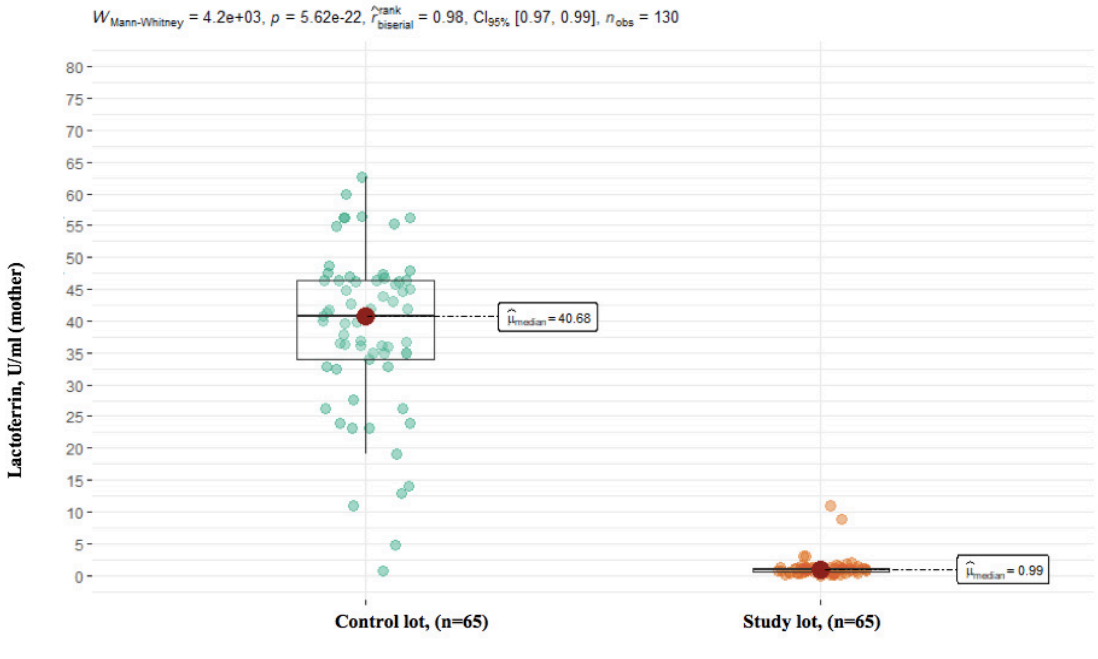

Fig. 1. Serum values of mother Lactoferrin in women with preterm delivery (control lot) and term delivery (study lot)

The study has shown that following the analysis, it was determined that in the study group (women with preterm spontaneous delivery) the level of *Lactoferrin* serum of women constituted in average 0.99 U/ml (median) being less in comparison to 40.68 U/ml (median) in the control group (women with term spontaneous delivery) (p<0.001) (fig. 1), effect size being large one ($r_{rank\ biserial}$=0.98 CI95% 0.97, 0.99). All these data show practical significance of the *Lactoferrin* serum oscillations in PTB group. Possibly, this phenomen is conditioned by the fact that *Lactoferrin* appears to play a critical role in the first line of host defense by modulating innate immune response. In connection with immune co-factors, maternal *Lactoferrin* modulates chemokines real to amplify host defense during pregnancy. *Lactoferrin* could interact with both maternal and fetal microenvironments to establish physical as well as immunological barriers to evade microbial pathogenesis.

Analysis of the immune mediators at the maternal-fetal interface revealed increased levels of proinflammatory *IL-6*. Thus, the data of this study demonstrated a significant increase in *Interleukine-6* values in the base group (women with preterm spontaneous delivery) – 51.90 pg/ml (median), compared to the control group (women with term delivery) – 21.51pg/ml, (median), (p<0.001), effect size being large one ($r_{rank\ biserial}$=0.85 CI95% -0.89, -0.78). The obtained results of the analysis show practical significance of the *IL-6* serum oscillations in PTB group.

At the same time, the correlational analysis was performed between *Lactoferrin* and *IL-6* in both groups in women with PTB and TB. Thus, the following results were obtained and are shown in table 1.

The serum levels of *Lactoferrin* were negatively associated with serum levels of *Interleukine-6* (-0.377 95%CI -0.580, -0.149, p=0.002) which denotes an alteration of the innate immune system by decreasing its defense capacity, represented by *Lactoferrin* possibly on the background of an asymptomatic infectious process, with the subsequent triggering of the inflammatory cascade and the secretion of pro-inflammatory cytokines such as *Il-6*. These active pro-inflammatory compounds induce the secretion of local prostaglandins and thrombin, decrease the concentration of progesterone, therefore there is an increase in uterine contractility, thus inevitably premature labor begins.

Table 1. The indicators of the molecular plasmatic profiles. Correlation's analysis (p Spearman) for the study group

	IL-6, pg/ml
Lactoferrin **U/ml**	-.377**
Sig. (2-tailed)	0.002
95% CI	-0.580
	-0.149

Legend: **Correlation is significant at the 0.01 level (2-tailed).

Conclusions

Preterm delivery remains a serious public health issue. Inflammation represents a major pattern related to several factors essential to triggering labor. However, the molecular triggers and mechanisms underlying the activation of immune pathways associated with induction of preterm delivery remain poorly understood.

Several types of pathogens that disseminate systemically or through the placenta play an important role in induction

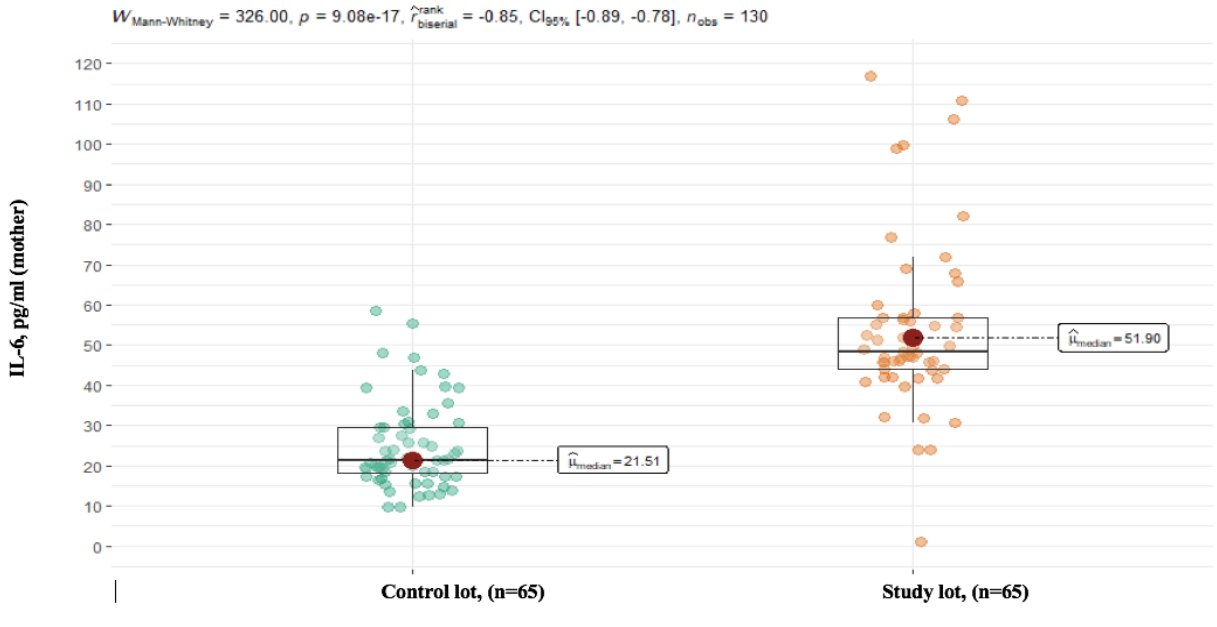

Fig. 2. Serum values of mother Interleukine-6 in women with preterm delivery (control lot) and term delivery (study lot)

of preterm delivery. The sensing of pathogen or endogenous ligand (uncovered during tissue injury and/or inflammation) by innate immune receptors and subsequent induction of immune mediators play an important role for shaping the phenotype and activity of various innate immune cells are known to participate in the labor process. Furthermore, such studies imply that a disruption of homeostasis, either systemically or at the maternal/fetal interface, by an infection and/or inflammatory triggers, contributes to adverse pregnancy outcomes. Therefore, it is important to mention the fact that medical screening of pregnant women for signs of infections and infection-associated immune mediators, such us, the serum levels of *Lactoferrin* and *Interleukine-6* thus may lead to the discovery of novel biomarkers, identify possible at-risk pregnancies, and help to define specific drugs (e.g., specific inhibitors and antibiotics, respectively) for an effective intervention.

Strengths and limitations

Many combined techniques and several compartments strengthened the obtained findings. By design, term pregnancies were used as controls because there is no ethical way to obtain gestational age matched tissues from healthy pregnancies. However, it was previously reported that the inflammatory changes associated with term physiological labor were significant when compared with those occurring in pathologic pregnancies, such as those with PTB.

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Authors' contributions

VC conceptualized the project, drafted the first manuscript and interpreted the data. CN, LM, MB added some conceptual ideas and corrected the text, LP, ID critically revised the manuscript. All the authors revised and approved the final version of the manuscript.

Ethics approval and consent to participate

The study was approved by the Research Ethics Committee of *Nicolae Testemitanu* State University of Medicine and Pharmacy, protocol No 16 of 21.11.2017.

Conflicts of interest. No competing interests were disclosed.

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