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ORIGINAL ARTICLE

The link between cord blood IL-1β, TLR4, PGE2 and TAC values with neonatal diseases

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Abstract

Premature birth is an important cause of neonatal mortality and neonatal morbidity. Most premature births are known to be induced by cytokines released for different reasons. Inadequate congenital immune response in premature infants may contribute to increased susceptibility to infection. The aim of the study is to determine the IL-1β, TLR4, PGE2, and TAC profiles in cord blood with characteristics specific to pregnancy and the correlation with neonatal complications caused by premature birth. The study included 26 neonates, 11 girls and 15 boys, born from 24-42 weeks of gestation. Of these, 13 were term and 13 were preterm . For IL-β, PG-E2, TLR4 and TAC levels, 1 mL of cord blood sample was taken from preterm and term neonates. Data related to demographic data, clinical status of patients and outcomes were obtained from electronic medical records and files. Cytokine values obtained from premature neonates were statistically high in terms of TLR4, IL1 and PGE2 compared to term infants. The TRL4 and IL1 values for premature infants with necrotizing enterocolitis and retinopathy of prematurity were lower compared to those without NEC and ROP. In spite of negative correlations between TAC and the other three cytokines, a statistically significant correlation was not identified. TLR4, IL1 and PGE2 were negatively correlated with weight and gestational week, contrarily TAC measurements were positively correlated with weight and gestational week. Measurements of cytokine concentrations in cord blood are among important biomarkers showing degree of inflammation and may assist in predicting neonatal complications and play an effective role in development of specific treatments.

Keywords: *Neonatal, umbilical cord blood, IL-1β, TLR4, PGE2, TAC*

Abbreviations: Necrotizing Enterocolitis (NEC), Retinopathy of Prematurity (ROP), Prostaglandin E2 (PGE2), Interleukin-1β (IL-1β), Reactive oxygen species (ROS), total antioxidant capacity (TAC), Toll-like receptor-4 (TLR4), Bronchopulmonary Dysplasia (BPD), Cord Blood Mononuclear Cells (CBMC), Tumor Necrosis Factor Alfa (TNF-α), Neonatal Intensive Care Unit (NICU), Enzyme Immunoassay (EIA), International Classification of Prematurity Retinopathy(ICROP)

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Introduction

Premature birth is an important cause of neonatal mortality and morbidity. Premature birth (<37 weeks) is a physiological process associated with negative outcomes like low birth weight (<2500 g) or very low birth weight (<1500 g) and comprises 5-10% of all births. Premature birth is linked to the maternal, fetal and placental causes. Repeated miscarriages, multiple births, placental defects, cervical and uterine anomalies, in vitro fertilization and polyhydramnios are risk factors for premature birth. Environmental factors like infection, alcohol, tobacco and low socioeconomic status are known to both increase systemic inflammation and the risk of premature birth. In order to provide a regular response to inflammation, immune cells, markers called cytokines or chemical mediators affecting other cells are released [1,2]. Cytokines released for different reasons are known to induce most premature births [2]. Contrary to a term neonate, preterm neonates have different cytokine and mediator profile levels and forms of control. These are shown as potential causes of increasing vulnerability to infections [1]. Increasing incidence of necrotizing enterocolitis (NEC), retinopathy of prematurity (ROP) and sepsis in premature infants are thought to be associated with cytokine profiles.

One of the bioactive mediators of inflammation is prostaglandin E2 (PGE2) and effects rapidly begin within hours. The half-life is short in most biological fluids; for example, t1/2 is 5 minutes in plasma. In spite of PGE2's basic role in the inflammatory phase, it is not routinely used in clinical practice due to accelerated metabolism and natural difficulty with calculations [4].

Pro-inflammatory cytokine interleukin-1β (IL-1β) is an active pyrogen and inflammatory mediator playing a role in many early diseases. After toll-like receptor activation, IL-1β develops as a precursor protein (pro-IL-1β) and after division comprises the multiple protein inflammatory complex of caspase 1 [5]. IL-1β is the main mediator of the congenital and adaptive immune system [6]. IL-1β is basically a soluble protein created by monocytes and macrophages. A significant increase is reported in IL-1β levels in response to infection, microbial toxins, inflammatory agents, complementary products in activated lymphocytes and clotting compounds [7].

Reactive oxygen species (ROS) are highly reactive molecules that may destroy lipids, proteins, polysaccharides and DNA during cellular digestion of carbohydrates and lipids for energy production. Antioxidant defense successfully prevents the negative effects of ROS under typical physiological conditions [8]. However, immature organ systems, predisposition to infection and inflammation, extra oxygen requirements and high free iron levels make premature infants susceptible to ROS [9]. In the last trimester of pregnancy, the antioxidant enzyme systems are basically up-regulated and increasing amounts of non-enzymatic antioxidants pass the placenta in the same time period [9]. This comprises the total antioxidant capacity (TAC). The approximate contribution of antioxidants to TAC is equivalent to sulfhydryl groups in free proteins (52.9%), uric acid (33.1%), ascorbic acid (4.7%), general bilirubin (2.4%), alpha tocopherol (1.7%) and others (5.2%). Results are expressed as mmol Trolox per liter equivalent.

Toll-like receptor-4 (TLR4) is a cell surface receptor on the cell wall recognizing gram negative bacterial compounds. Premature birth is among diseases triggered by irregular activation of TLR4 to bacterial endotoxins. Diseases like neonatal sepsis, bronchopulmonary dysplasia (BPD), NEC and ROP specific to premature infants that may form as a result of premature birth do not have effective pharmacological medications [11]. The neonatal cord blood mononuclear cells (CBMC) have less tumor necrosis factor (TNF-α) and IL-1β in response to stimulation of the TLR ligand compared to adult cells; however, they release more IL-10 and IL-6 [12]. Stimulation of TLR by microbial ligands causes the necessary range of responses for inflammatory induction and adaptive immunity leading to activation of many signal pathways [12]. A variety of studies were performed with cytokines, the key agents in intrauterine inflammation and associated neonatal complications. Cytokine concentration levels in cord blood, amniotic fluid and neonatal peripheral blood may reflect the degree of inflammation and may assist in predicting neonatal conditions [13]. The aim of this study was to research the association between IL-1β, TLR4, PGE2, and TAC profiles in cord blood of term and preterm neonates with demographic features, need for admission to neonatal intensive care unit (NICU), and the neonatal complications caused by premature birth of NEC, ROP and sepsis.

Materials and Methods

Study Plan

This prospective research performed in a hospital with 17-bed tertiary NICU was completed from July 2019 to December 2020. The research included a total of 26 neonates (11 girls and 15 boys). The study

included neonates born between 24 and 42 weeks of gestation. Of these, 13 were term (>37 weeks) and 13 were preterm (<37 weeks). The research excluded patients with known or suspected congenital heart abnormalities, genetic disorders, metabolic disease and other congenital anomalies. Patients who did not require clinical blood sampling for sample collection were excluded from the study. Demographic variables, NEC, sepsis, ROP and Apgar scores were obtained from data collected. Information related to the mother was retrospectively obtained from the mother's medical history. The study was permitted by by Usak University Ethics Committee 11,07,2019/213-07 and informed consent was obtained from families. The study was performed in accordance with the Declaration of Helsinki Basil protocol 2013.

Blood Sample Collection and Cytokine measurement

Umbilical venous samples of 1 ml were obtained for preterm and term babies for IL-β, PG-E2, TLR4 and TAC serum, isolated by centrifugation for 10 minutes at 1500 rpm. Samples of serum is deposited at-80 ° C. TAC measurement of cord blood samples was done with manufacturer's kit protocol (Rel Assay Diagnostics kit; Turkey) which is based on reducing ın the samples which include antioxidants, dark blue green colored ABTS radical to colorless ABTS type. In this assay, TAS levels were colorometrically detected the change of absorbance at 660nm using microplate reader. (Thermo Fisher Scientific, Finland). The results were indicated as μmol Trolox equivalent/L (μmolTroloxeq/L) [14] .

The levels of cord blood TLR-4, PGE-2, and IL-1β (USCN Life Science, Wuhan, China) were measured by enzyme immunoassay (EIA). Absorbance was carried out on Thermo fisher Multi Sky System (Thermo Fisher Scientific, Finland). Cord blood concentrations of TLR-4, PGE2, and IL-1β were determined from a curve obtained with the standards. The package detection limits were 0.118 ng /ml and 8.43pg/ml respectively, for TLR-4 and PGE-2, and 8,43pg/mlfor IL-1β according to manufacturer's instruction

Data Processing for Patients

Neonatal data from electronic medical reports, including demographic data, clinical factors and outcomes, were reported. Apgar scores were recorded one and five minutes after birth. Patients were separated into two on the basis of APGAR scores: <7 and ≥7 [15]. All of our 6 NEC diagnosis patients were premature and 4 were evaluated as suspected necrotizing enterocolitis and 2were Conclusive medical necrotizing enterocolitis according to the modified bell criteria [16]. In 7 premature patients, the diagnosis of ROP was made according to ICROP (International Classification of Prematurity Retinopathy) as a result of regular eye exams conducted in our clinic. 4 patients Stage 1: vascular and avascular retina demarcation line, 2 patients Stage 2: back (ridge); surface swelling, and 1 patient confirmed to be Stage 3: fibrovascular extraretinal (fibrovascular) proliferation at the back [17]. Sepsis has been diagnosed in 8 patients with a positive, urine, CSF or blood culture (> 72 hours) [18]. All newborns admitted to the research center's intensive care unit underwent screening echocardiography examination within 24 hours of their admission. A systematic echocardiographic cross-sectional and doppler test was conducted with several parasternal, suprasternal, apical and subcostal orthogonal views with 6-MHz transducers (Vivid S6, GE Healthcare, UK). Six patients were diagnosed with PDA by transthoracic echocardiography.

Statistical Analysis

Descriptive statistics in the tables are given as mean and standard deviation, and median and interquartile range. As a result of the Kolmogorov-Smirnov test, the statistical distribution of cytokines values was not suitable for normal distribution in any of the groups compared. The Mann Withney U test was used both for the comparison of term and preterm and for comparisons with and without Nec, Rop and Sepsis within the preterm group. However, it was used in all of the other two group comparisons. Relationships between cytokines, week and weight were determined with the Spearman correlation coefficient. In G*Power software, For all comparisons with a statistically relevant minimum $p<0.05$, effect sizes and post-hoc power analysis were conducted for. The minimum and maximum effect sizes were calculated to be 0.673-2.41 in the study. The strength of the test was estimated at a minimum of 49.24% and a maximum of 99.9%. For statistical analysis, SPSS software for Windows version 25.0 (Statistical Package for Social Sciences Inc, Chicago, IL, USA) was used.

Results

Among cytokine values, the TAC value was found to be low by a statistically significant degree in premature infants ($p<0.05$). The cytokine values obtained from premature neonates in terms of TLR4, IL1 and PGE2 were found to be statistically significantly high compared to term infants ($p<0.01$). The IL1 value

especially was four times higher in preterm neonates compared to term neonates. The descriptive statistics and statistical comparisons in terms of cytokine values of preterm and term infants are given in Table 1.

Figure 1 gives the comparison of cytokines and TAC in premature neonates in terms of those with and without NEC and ROP. Due to the low number of observations and variability, comparisons used the Mann Whitney U test. All of the investigated cytokines were not statistically significant in terms of premature neonates with/without NEC and ROP (p>0.05). The TAC and PGE2 values of those with and without NEC and ROP were very close to each other. Additionally, the TLR4 and IL1 values were found to be higher in those without NEC and ROP compared to those with these conditions. Though statistically insignificant, this difference in IL1 levels was two times more than those without these conditions.

Table 2 gives the comparisons of cytokines in preterm and term neonates in terms of sepsis, gender, NICU admission, Apgar score, type of birth, smoking and patent ductus arteriosus (PDA). The IL1 and PGE2 values of neonates with sepsis had statistically higher values compared to those without sepsis (p<0.05). In terms of gender, being a female or male infant did not appear to cause a change in cytokine values (p>0.05). In terms of need for NICU admission or not, TLR4 levels were not different (p>0.05), while neonates requiring NICU admission had statistically significant degree of elevation in IL1 and PGE2 levels, while TAC levels were significantly low (p<0.05). Apgar scores above or below 7, birth type of vaginal or cesarean, and smoking habit or not did not cause statistically significant differences in terms of cytokines (p>0.05). In the presence of PDA, only PGE2 levels were found to be high by a statistically significant degree (p<0.05).

Table 1. Comparison of cytokine levels in cord blood of term and preterm infants

	Preterm		Term		
Variables	Mean±S.D.	Median(IQR)	Mean±S.D.	Median(IQR)	p value*
TAS	0.90±0.06	0.88(0.08)	0.99±0.13	0.97(0.12)	0.026
TLR4	2.79±0.55	2.89(0.54)	2.24±0.39	2.11(0.40)	0.009
IL1	228.25±252.6	112.92(248.88)	53.77±55.52	30.39(67.52)	0.007
PGE2	1072.25±84.73	1084.37(163.13)	726.38±722.23	620.24(735.86)	0.009

*:MannWithneyU p value, S.D.:Standard deviation, IQR: interquartile range

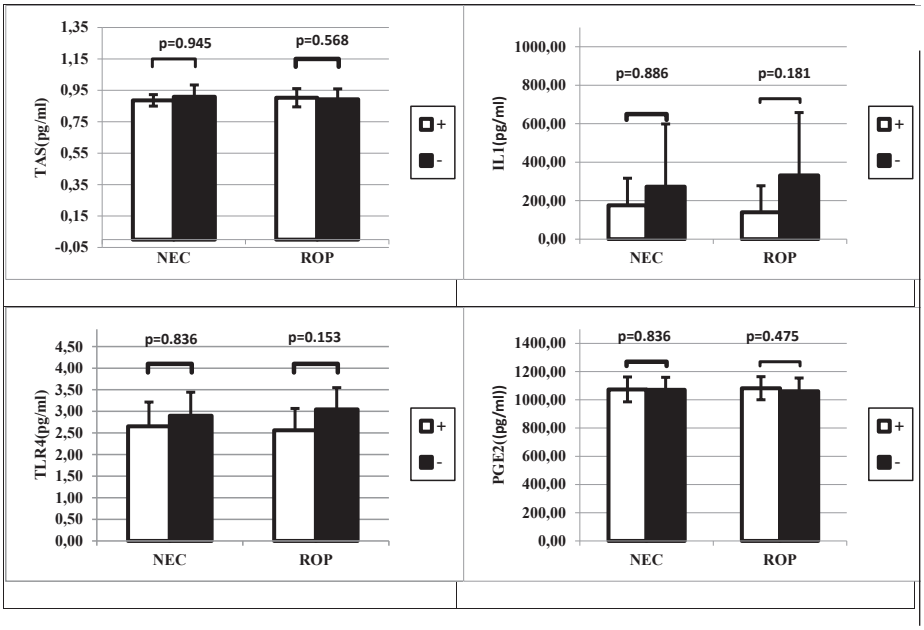

Figure 1. Comparison of cytokinins in the Premature group in terms of ROP and NEC (+:existence, - : non-existence)

Table 2. Comparison of TLR4, PGE2, IL1B and TAS values in terms of perinatal characteristics of newborns

Variables		Cytokines			
		TLR4	**IL1**	**PGE2**	**TAS**
		Median(IQR)	**Median(IQR)**	**Median(IQR)**	**Median(IQR)**
Sepsis	+	2.79(0.51)	112.92(112.76)	1015.69(163.13)	0.89(0.07)
	-	2.16(0.84)	32.68(74.42)	758.73(648.88)	0.96(0.11)
	pvalue	0.0765	**0.0165**	**0.0315**	0.1235
Gender	**Male**	2.16(0.9)	102.39(178.25)	984.47(381.85)	0.91(0.12)
	Female	2.62(0.65)	73.47(75.73)	1015.69(788.19)	0.96(0.1)
	pvalue	0.337	0.622	0.979	0.815
Need For NICU Admission	+	2.62(0.92)	112.92(288.9)	1015.69(175.22)	0.88(0.12)
	-	2.31(0.95)	32.68(65.2)	620.24(753.04)	0.97(0.08)
	pvalue	0.1435	**0.026**	**0.0365**	**0.041**
APGAR	**Down7**	2.89(1.19)	147.25(307.87)	1084.37(190.26)	0.88(0.07)
	Up7	2.33(0.85)	57.84(77.69)	990.52(651.66)	0.96(0.13)
	pvalue	0.063	0.107	0.156	0.0965
Delivery	**NSVD**	2(1.16)	30.39(339.53)	758.73(281.09)	1.1(0.49)
	C/S	2.44(0.88)	82(122.55)	1004.29(686.81)	0.91(0.1)
	pvalue	0.1995	0.2735	0.2475	0.1395
Tobacco	**Smoking**	2.98(1.35)	112.92(297.75)	1015.69(149.36)	0.9(0.11)
	Non-Smoking	2.4(0.87)	73.47(130.45)	984.47(676.17)	0.91(0.11)
	pvalue	0.446	0.316	0.399	0.399
PDA	+	2.61(1.05)	117.85(221.93)	1107.83(69.28)	0.91(0.14)
	_	2.42(0.87)	53.46(104.07)	981.45(643.36)	0.91(0.1)
	pvalue	0.165	0.081	**0.0255**	0.4755

IQR: interquartile range

Table 3. Spearman correlation matrix between cytokines, weight and weeks

	IL1	PGE2	TAS	Weight	Week
TLR4	0.565**	0.305	-0.229	-0.459*	-0.544**
IL1	1	0.554**	-0.352	-0.488*	-0.597**
PGE2		1	-0.043	-0.343	-0.402*
TAS			1	0.392*	0.495*
Weight				1	0.904**

* and ** symbols shows correlation are significant at the 0.05 and 0.01 levels respectively.

Table 3 gives the correlation coefficients and statistical significance of cytokines in correlation with weight (kilo) and week of gestation. When the correlations between cytokines and TAC levels are examined, TLR4 and IL1 had 56.5% positive correlation and IL1 and PGE2 had 55.4% positive correlation and these were statistically significant ($p<0.01$). Though there were negative correlations between TAC levels with the other three cytokines, statistically significant correlations were not present. TLR4, IL1 and PGE2 were negatively correlated with weight and week of gestation ($p<0.05$, $p<0.01$). Contrary to this, TAC levels had a positive correlation with weight and week of gestation ($p<0.05$).

Discussion

In our study, the effect or not of TLR4, PGE2, and IL-1β cytokine levels and TAC levels on early birth was investigated along with the connections between sepsis, gender, need for admission to neonatal intensive care unit, APGAR score, type of birth, smoking and PDA development with cytokine profiles. Simultaneously cytokine profiles measured in cord blood were examined for whether they could be markers for NEC, ROP and sepsis and correlations between the cytokines were compared.

Cytokines are known to play roles in placental development and birth, as much as in the immune system [2]. In our study, TLR4, IL-1β and PGE2 concentrations in cord blood of premature neonates were found to be high by a statistically significant degree compared to values in the cord blood of term infants (Table 1). A study by Huang et al. associated the correlation between cord blood levels of phagocyte mediators IL-8 and MPO and vascular mediators of PGE2 and sVCAM-1 with differences in fetal maturity with gestational age. While higher IL-8 and MPO levels were identified in preterm neonates, PGE2 levels were low; however, there was no correlation with gestational age [19]. In our study, PGE2 levels were higher in cord blood of premature neonates, while there was a negative correlation with week of pregnancy. When the physiological features of premature and term neonates and pharmacological properties of PGE2 are compared, our results are compatible with the features of premature neonates and PGE2 properties.

In our study, TLR4, IL-1β and PGE2 concentrations in the cord blood of premature neonates were found to be negatively associated with gestational age and weight (Table 4). Comparisons between groups according to gestational week by Otsubo et al. revealed the inflammatory cytokine levels of IL-6, IL-1β, IL-1ra, and IL-13 and chemokine MIP-1β levels were significantly higher in groups from 37-41 weeks and less than 32 weeks compared to the group from 32-36 weeks. In our study, a negative correlation was identified between week and IL1-β, with IL1-β levels reducing as the gestational week increased. In our study, neonates below and above 37 weeks were compared and the IL1-β level was high in the group below 37 weeks and low in the group above 37 weeks. In the study by Otsubo et al. three groups were compared, with separate assessment of the group below 32 weeks with high IL1-β and the 32-36 week group with low IL1-β. When the 32-36 week and below 32 week groups are compared, results compatible with our study emerge [20].

In our study, premature infants were found to have four times higher IL-1β cord blood levels compared to term infants and there was a negative correlation between IL-1β levels with gestational week. In our study, the TLR4 levels in preterm cord blood were statistically higher compared to term infants and there was a negative correlation with gestational week. A study by Robertson et al. identified a negative correlation between gestational week with TLR4, as in our study. They recommended the use of TLR4 antagonists for inhibition of fetal, placental and intraamniotic inflammatory cytokine production to prevent premature birth. The results of our study support this proposal [21]. Additionally, Shen et al. found a reduction in toll-like receptor 2 (TLR2) and TLR4 surface expression in blood monocytes of premature neonates [22]. Mathias et al. reported a significant increase in interleukin 10 (IL-10) levels in cord blood after 24-hour ex vivo TLR4 stimulation [23].

Similar studies were performed with different body fluids. A study not using cord blood found urine PGE2 levels were high in premature infants and there were higher rates of increase in the first days of life in premature infants compared to term infants [24]. A study by Yoon et al. associated the elevation in IL-1β levels in amniotic fluid, but absence in cord blood, with intrauterine infection and premature birth [25]. In our study, though statistically significant differences could not be shown between term and preterm infants for IL-1β levels in cord blood, higher levels were identified in the cord blood of preterm infants. This situation leads to the consideration that preterm birth may be associated with intrauterine infections and intrauterine infections induce premature birth. A study by Narendran et al. found higher IL-1β levels

on preterm skin (skin surface of preterm infants) compared to term, adult and vernix, similar to our study [26].

In our study, TAC values measured in cord blood were observed to be low by a statistically significant degree in premature neonates compared to term neonates ($p<0.05$). Positive correlations were found between TAC value with gestational week and weight ($p<0.05$). Huertas et al. [27] found high concentrations of hydroperoxide in the erythrocyte membranes at birth and in the first days of life of premature infants in their study. They identified that the erythrocyte membranes of premature neonates were at much lower levels and/or had lower antioxidant defense functions compared to term neonates [27]. A study by Dizdar et al. observed lower TAC levels in infants younger than 28 weeks compared to those over 28 weeks [28]; similarly, in our study, TAC levels were identified to be low in the cord blood of premature infants. Both studies show that the antioxidant defense mechanism of small infants is immature. A study of premature infants by Georgeson et al. found cord blood antioxidant enzyme activities were lower compared to term infants, similar to our study, while cord blood lipid peroxidation markers were similar in term and preterm infants [29].

In our study, statistically higher IL1 and PGE2 values were identified in patients with sepsis compared to those without sepsis ($p<0.05$) (Table 2). Siljehave et al. mentioned increases in prostaglandin E2 (PGE2) induced with proinflammatory cytokine interleukin IL-1β infection and disrupted respiration as a result of this increase. In our study, both PGE2 and IL-1β levels were increased in infants with sepsis which is compatible with the literature and this study [4].

Similar to our study, a study related to sepsis using cord blood by Santana et al. found IL-8 concentration in cord blood was the most sensitive cytokine for early neonatal sepsis [30]. Some studies identified an increase in TNF-α in cord blood with early neonatal sepsis [31]. Contrary to our study, a study by Atıcı et al. showed IL-1 concentrations significantly reduced in preterm and term neonates with sepsis [32]. Ozdemir et al. found no significant difference in serum IL-1β levels in septic neonates compared to healthy controls [31].

In our study, there was no statistical difference between cord blood TLR4 levels in neonates with and without sepsis. The study by Shen et al. showed a reduction in surface expression of TLR2 and TLR4 in blood monocytes of premature neonates and a lower response to lipopolysaccharide (LPS) stimulation compared to TNF-α and IL-8 [22]. In our study, TLR4 was negatively correlated with gestational week. In other words, compared to term infants, preterm infants were identified to have higher rates of TLR4. A study by Levy et al. stated increased TLR4 and CD14 expression with stimulation of neonatal monocyte LPS may cause flare ups of normal inflammation during severe infection [33].

In our study, neonates with PDA were found to have statistically high PGE2 cord blood levels ($p<0.05$) (Table 2). Prostaglandins, especially PGE2, play an important role in maintaining the patency of fetal PDA [34].

As a result of analyses, type of birth, in other words spontaneous vaginal birth or cesarean birth, did not cause a statistically significant difference in terms of TLR4, PGE2, IL-1β and TAC. A study by Steinborn et al. showed that TNF-α and IL-1β levels in spontaneous vaginal birth cases were much higher compared to cesarean births [35]. In our study, the reason for the lack of statistically significant difference is thought to be the small sample size. A study by Hasegawa-Nakamura et al. observed that vaginal birth can induce TLR4 expression on monocytes and that lower receptor levels were seen in infants born by cesarean section [36]. Molloy et al. reported that in cesarean neonates, neonatal neutrophils had higher TLR4 expression compared to neonates born by the vaginal route [37].

When neonates in our study are assessed in terms of NICU admission requirements, there was no statistically significant difference in TLR4 levels ($p>0.05$). Statistically significant elevations in IL-1β and PGE2 and statistically significant fall in TAC values were identified in neonates requiring admission to hospital ($p<0.05$) (Table 2). Dizdar et al. [28] analyzed the association between TAC and total oxidative stress (TOS) levels of infants admitted to the NICU and healthy infants and similarly found low TAC levels were associated with longer duration of respiratory support and hospitalization [28].

In our study, the TAC levels and PGE2 values of premature infants with and without NEC and ROP were observed to be very close. However, TLR4 and IL2 values were higher in patients without NEC and without ROP. Though statistically insignificant, this difference was two times higher for IL-1β (Figure 1). Goepfert et al. showed increased IL-6 levels in umbilical blood samples and increased NEC risk in premature infants [38]. Satar et al. identified that

premature birth and NEC were associated with IL-8 in cord blood [39]. Yazji et al. showed disrupted neonatal intestinal perfusion in the NEC pathogenesis with TLR4 activation in endothelium. In experimental NEC induced in rats deficient in TLR4, mucosa inflammation and intestinal necrosis were reduced [40]. In our study, the basic reason for the lack of significant difference is the small sample size. We think developing this study with larger sample groups will identify a significant difference.

Conclusion

Preterm infants were found to have higher IL-1β values, especially, along with TLR4 and PGE2 levels compared to term infants. Among preterm infants, those with ROP and NEC were found to have higher IL-1β values, by two times, and TLR4 values. It is considered that if samples are taken from cord blood and studied before the development of NEC and ROP, IL-1β and TLR4 antagonists can be administered with the threat of early birth and both premature birth and NEC and ROP development may be prevented. This is supported by literature information (21). Additionally, design of studies administering IL1 and TLR4 antagonists in the early stages of NEC and ROP development in larger sample groups will form an important stage for the transition to treatment.

IL1 and PGE2 levels were higher in septic neonates. Additionally, infants admitted to the NICU were identified to have higher IL1 and PGE2 levels. The use of IL1 antagonists for sepsis treatment may be promising and cheering.

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Conflict of interest

The authors have notified that no competing interests conflict and published at the stage of preparation of this manuscript.

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