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RESEARCH

How does being a twin premature infant affect systemic inflammatory indices?

İkiz prematüre bebek olmak sistemik inflamatuvar indeksleri nasıl etkiler?

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Abstract

Purpose: Systemic inflammatory indices are newly defined parameters in diseases in the field of neonatology. However, it is not known whether the levels of systemic inflammatory indices change in twin preterms.

Materials and Methods: Premature infants <32 weeks of gestational age were included in our study. Demographic characteristics, morbidity, mortality, leukocyte, neutrophil, monocyte, lymphocyte, platelet count data of all patients were recorded retrospectively. Neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), monocyte-to-lymphocyte ratio (MLR), systemic immune ratio-inflammation index (SII), pan-immune-inflammation value (PIV) and systemic inflammation response index (SIRI) values were calculated. Twins and singleton infants were compared with each other in terms of demographic characteristics, morbidity, mortality, complete blood count parameters and systemic inflammatory indices.

Results: A total of 953 infants, including 200 twins and 753 singleton premature infants, were included in the study. Cesarean section, respiratory distress syndrome, and intraventricular hemorrhage were found to be higher in the twins group compared to the singleton group. The leukocyte, neutrophil, monocyte, lymphocyte count was found to be lower in the twins group and the platelet count was found to be significantly higher in the singleton group. PLR value was higher (41.81 and 32.09, respectively) and PIV value was lower (43.66 and 47.93, respectively) in the twins group compared to the singleton group. NLR, MLR, SII, SIRI values were similar between twins and singleton groups. There was no difference in demographic characteristics, morbidity, mortality, complete blood count parameters and systemic inflammatory indices of the first and the second twin premature infants.

Conclusion: Differences in systemic inflammatory indices and multiple hematological parameters between twins and singleton premature infants should be interpreted carefully

Öz

Amaç: Sistemik inflamatuar indeksler neonatoloji alanında hastalıklarda yeni tanımlanan parametrelerdir. Ancak ikiz prematürelerde sistemik inflamatuar indeks düzeylerinin değişip değişmediği bilinmemektedir.

Gereç ve Yöntem: Çalışmamıza gebelik yaşı <32 hafta olan prematüre bebekler dahil edildi. Tüm hastaların demografik özellikleri, morbidite, mortalite, lökosit, nötrofil, monosit, lenfosit, trombosit sayı verileri geriye dönük olarak kaydedildi. Nötrofil-lenfosit oranı (NLR), trombosit-lenfosit oranı (PLR), monosit-lenfosit oranı (MLR), sistemik immün inflamasyon indeksi (SII), pan-immün-inflamasyon değeri (PIV) ve sistemik inflamasyon yanıt indeksi (SIRI) değerleri hesaplandı. İkiz ve tekil bebekler demografik özellikler, morbidite, mortalite, tam kan sayımı parametreleri ve sistemik inflamatuar indeksler açısından birbirleriyle karşılaştırıldı.

Bulgular: Çalışmaya 200'ü ikiz, 753'ü tekil prematüre olmak üzere toplam 953 bebek dahil edildi. İkiz grupta sezaryen doğum, solunum sıkıntısı sendromu ve intraventriküler kanama tekil gruba göre daha yüksek bulundu. İkiz grupta lökosit, nötrofil, monosit, lenfosit sayısı daha düşük, trombosit sayısı ise tekil grupta anlamlı olarak yüksek bulundu. İkiz grupta tekil gruba göre PLR değeri (sırasıyla, 41,81 ve 32,09) daha yüksek, PIV değeri (sırasıyla, 43,66 ve 47,93) daha düşüktü. NLR, MLR, SII, SIRI değerleri ikiz ve tekil gruplar arasında benzerdi. Birinci ve ikinci ikiz prematüre bebeklerin demografik özellikleri, morbidite, mortalite, tam kan sayımı parametreleri ve sistemik inflamatuar indeksleri açısından farklılık yoktu.

Sonuç: İkizler ve tekiz prematüre bebekler arasındaki sistemik inflamatuar indeksler ve çoklu hematolojik parametrelerdeki farklılıklar klinik sonuçları anlamak için pre/postnatal koşullar değerlendirilerek dikkatle yorumlanmalıdır.

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taking into account pre/postnatal conditions to understand clinical outcomes.
Keywords:. Systemic inflammatory indices; infant; premature; singleton; twin

Anahtar kelimeler: : Sistemik inflamatuar indeksler; yenidoğan; prematüre; tekil; ikiz

INTRODUCTION

In recent years, the prevalence of twin pregnancies has increased due to the prominence of infertility treatments. Along with the increase in premature twin births, the frequency of hospitalization in the neonatal intensive care unit (NICU) also increases. As the standards of care of premature babies improve, so does their chance of survival. The higher rate of survival of more premature infants increases the morbidity and mortality due to prematurity[1]. Increased morbidity and mortality is primarily associated with low gestational age (GA) and birth weight. Complete blood count (CBC) parameters used to evaluate the clinical status of premature infants can provide information about morbidity and mortality. The difference between twins or singleton infants in terms of morbidity and mortality can be explained by the difference in CBC parameters[2].

Hemoglobin, hematocrit, nucleated red blood cells, platelets, total leukocyte, neutrophil, lymphocyte, monocyte, eosinophil, and basophil count may differ between singleton and twins, and between the first and the second twins as well. These differences in blood counts may affect clinical outcomes both between singleton and twins and between first and second twins[2,3]. Therefore, it is necessary to better analyze and interpret hematological parameters in order to understand the clinical outcomes of premature infants who are quite fragile[2].

Systemic inflammatory indices calculated using the neutrophil, lymphocyte, monocyte, and platelets count have recently come into clinical use. It has been stated that systemic inflammatory indices may be significant in determining the severity of diseases and predicting clinical outcomes in some adult and pediatric diseases[4,5]. In the field of neonatology, it has been reported that systemic inflammatory indices can be useful parameters for evaluating the diagnosis and outcomes of certain diseases such as hypoxic ischemic encephalopathy (HIE), sepsis, retinopathy of prematurity (ROP), intraventricular hemorrhage (IVH)[6-9]. It is known that complete blood count parameters may differ between singleton and twin, and between the first and the second twins. However, it is not known whether systemic inflammatory indices vary between singleton and twins, and between the first and the second twins, especially in premature infants. According to our hypothesis, systemic inflammatory indices may differ both between singleton and twins and between the first and the second twins. This difference may play a role in differing clinical outcomes between singletons and twins. As far as we know, there is no study conducted for this purpose in the literature. Therefore, in our study, singleton and twin infants <32 weeks of GA were compared among themselves in terms of hematological parameters and systemic inflammatory indices.

MATERIALS AND METHODS

Study design

All infants admitted to NICU in Zekai Tahir Burak Maternity Training and Research Hospital between February 2019 and April 2020 were analyzed retrospectively. Premature infants <32 weeks of GA were included in the study. Infants ≥32 weeks of GA or <32 weeks of gestational age infants with congenital anomalies, Down syndrome, hematological disorders, twin–twin transfusion syndrome (TTTS) and triplets were not included in the study. The study protocol was started after obtaining approval from the local ethics committee (Zekai Tahir Burak Maternity Training and Research Hospital Clinical Research Ethics Committee, no: 51/2019, date: 11.4.2019).

All data were obtained from hospital medical records and evaluated retrospectively. To ensure the reliability of the records and to protect the privacy of the patients, all data were kept confidential and not shared anywhere. All stages of the study were carried out by two specialist physicians. The same treatment protocols were applied to all patients throughout the study period. 953 premature infants were included in the study and analyzed. 753 premature infants were enrolled in the singleton group and 200 premature infants in the twins group (100 pairs of premature). All infants were born in our hospital. In our clinic, delayed cord clamping is applied if the premature infant's clinic was suitable at the time of birth and there is no obstacle to cord clamping. The same

protocol was applied to all patients during the study period.

Demographical features and clinical outcomes in premature infants

GA, birth weight (BW), administration of antenatal steroid, gender, mode of delivery, chorioamnionitis, Apgar scores at the 1st and 5th minutes, duration of mechanical ventilation (MV) support, early onset sepsis (EOS), late onset sepsis (LOS), respiratory distress syndrome (RDS), severe IVH, patent ductus arteriosus (PDA), necrotising enterocolitis (NEC), bronchopulmonary dysplasia (BPD), ROP, duration of hospitalization, mortality and CBC results were recorded.

Definition of preterm morbidities

Neonatal sepsis was defined as EOS in the first 3 postnatal days and as LOS after the postnatal 3rd day[10]. Infants given surfactant were recorded as having RDS[11]. If PDA was detected in a premature infant with Doppler echocardiography together with clinical findings and received medical treatment at least once, it was defined as PDA[12]. If NEC is defined as moderate or advanced (stage ≥2), it is registered[13]. According to BPD classification; those who still needed <30% oxygen at the 36th week of postmenstrual age were defined as moderate and those who received positive pressure respiratory support or ≥30% oxygen support were defined as severe BPD[14]. Infants who were found to have retinopathy in the retinal scan and received laser treatment were registered as ROP[15]. Infants with grade ≥3 IVH detected by cranial ultrasonography (severe IVH) were recorded[16].

Analysis of complete blood count

Venous blood samples were taken from the premature infants within the first hour after birth, and placed in ethylenediaminetetraacetic acid (EDTA) tubes for complete blood count. CBC analysis was performed by using the Cell-Dyn 3700 automatic hemocytometer (Abbott, Abbott Park, IL, USA). Leukocyte count (10^3 μ/L), neutrophil (N) count (10^3 μ/L), monocyte (M) count (10^3 μ/L), lymphocyte (L) count (10^3 μ/L), and Platelet (P) count (10^3 μ/L) data were recorded. C-reactive protein (CRP), and interleukin-6 (IL-6) levels were recorded.

Calculation of systemic inflammatory indices

Neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), monocyte-to-lymphocyte (MLR), systemic immune ratio-inflammation index (SII), pan-immune-inflammation value (PIV), and systemic inflammation response index (SIRI) values were evaluated as systemic inflammatory indices. N, M, L, and P counts were used to calculate systemic inflammatory indices. NLR; N count divided by L counts, PLR; P count divided by L count, MLR; M count is obtained by dividing by L count, PIV, P count, N count, and M count are multiplied by L count, SII is obtained by dividing P count by N count and dividing by L count, and SIRI is obtained by multiplying N count by M count and dividing by L count[6].

Patients <32 weeks of GA and meeting the study criteria were first divided into two groups as the singleton and the twins. Then, twin premature infants were again divided into 2 subgroups as the first and the second twin. The groups were compared in terms of demographical features and clinical outcomes, preterm morbidities and mortality, results of complete blood count, and systemic inflammatory indices.

Statistical analysis

Statistical Package for Social Sciences (SPSS), version 20.0 (SPSS Inc, Chicago, IL, USA) analysis program was used for statistical analysis. Histogram and Kolmogorov-Smirnov Test were used to examine the distribution of the variables. Fisher's Exact test or Pearson Chi-Square test was used in the analysis of the categorical variables, and t test or Mann-Whitney U test was used in the analysis of the continuous variables. Normally distributed continuous variables were presented as mean ± standard deviation, and non-normally distributed variables were presented as median and interquartile range (IQR). The results of categorical variables were presented as frequency. The area under the curve (AUC) and cut-off values were calculated using the ROC analysis. A p value of <0.05 was defined as significant. Calculation of the sample size was performed with G-Power Version 3.1.9.6. Statistical power was calculated before data collection based on information from previous studies[17,18]. Considering twins and singletons, it was found that at least 162 patients (81 patients per group) were required, with an effect size of 0.50, type I error of 0.05, and power of 0.80.

RESULTS

997 premature infants <32 weeks of GA were followed up in the NICU. A total of 44 infants, including 7 with congenital anomalies, 1 with down syndrome, 1 with hematological disorders, 8 with TTTS, and 27 triplets, were not included in the study. The remaining 953 premature infants were included in the study and analyzed. 753 premature infants were enrolled in the singleton group and 200 premature infants in the twins group (100 pairs of premature).

GA, BW, administration of antenatal steroid, male gender, chorioamnionitis, Apgar scores at the 1st and 5th minutes, duration of MV, EOS, LOS, PDA, NEC, BPD, ROP, duration of hospitalization, and mortality were similar between singleton and twins patients ($p>0.05$). Cesarean section, RDS, and IVH rates were found to be statistically significantly higher in the twins group compared to the singleton group ($p<0.001$, $p<0.001$, and $p=0.005$, respectively) (Table 1). In the twins group, leukocyte, neutrophil, monocyte, lymphocyte count was lower than those in the singleton group, but the platelet count in the twins was found to be significantly higher compared to singletons ($p=0.042$, $p=0.012$, $p=0.038$, $p=0.049$, and $p=0.034$, respectively). The PLR value was higher and the PIV value was significantly lower in the twins group compared to singleton group ($p<0.001$ and $p=0.014$, respectively). The results of CRP, IL-6, NLR, MLR, SII, and SIRI values were similar between the groups ($p>0.05$) (Table 2).

Table 1. Demographic characteristics and clinical outcomes between twins and singletons

Characteristics	Singletons (n=753)	Twins (n=200)	P value
Gestational age, weeks a	28.1±1.2	28.1±1.1	0.680
Birth weight, g a	1057±227	1110±203	0.053
Antenatal steroid, n (%)	651 (70.1)	128 (64)	0.731
Male gender, n (%)	366 (48.6)	105 (52.5)	0.329
Cesarean section, n (%)	610 (81.0)	192 (96.0)	<0.001*
Chorioamnionitis, n (%)	113 (15.0)	32 (16.0)	0.611
Apgar 1st min, b	6 (1)	5 (2)	0.089
Apgar 5th min, b	8 (1)	7 (2)	0.057
Duration of MV, days b	1 (3)	1 (4)	0.058
EOS, n (%)	13 (1.7)	2 (1)	0.390
LOS, n (%)	173 (22.9)	36 (18.0)	0.070
RDS, n (%)	450 (59.7)	149 (74.5)	<0.001*
IVH, n (%)	56 (7.4)	25 (12.5)	0.005*
PDA, n (%)	271 (35.9)	74 (37.0)	0.793
NEC, n (%)	19 (2.5)	4 (4.0)	0.080
BPD, n (%)	115 (15.2)	35 (17.5)	0.496
ROP, n (%)	78 (10.3)	22 (11.0)	0.145
Duration of hospitalization, days, b	54 (36)	55 (29)	0.392
Mortality, n (%)	72 (9.5)	21 (10.5)	0.294

[a] mean ± standard deviation, [b] median (interquartile range); *P<0.05 was considered statically significant.

Table 2. Systemic inflammatory indices in twins and singletons

Parameters	Singletons (n=753)	Twins (n=200)	P value
Leukocyte count (103 μ/L) a	11.60 (9.10)	9.30 (7.68)	0.042*
Platelet count (103 μ/L) a	227.00 (102.00)	228.00 (119.00)	0.034*
Neutrophil count (103 μ/L) a	2.35 (2.59)	2.10 (1.98)	0.012*
Monocyte count (103 μ/L) a	0.71 (0.67)	0.61 (0.52)	0.038*
Lymphocyte count (103 μ/L) a	7.58 (6.55)	6.63 (4.97)	0.049*
NLR a	0.31 (0.39)	0.31 (0.29)	0.401
MLR a	0.09 (0.06)	0.09 (0.05)	0.641
PLR a	32.09 (28.10)	41.81 (36.30)	<0.001*
PIV a	47.93 (54.40)	43.66 (51.85)	0.014*
SII a	73.63 (73.97)	81.79 (73.33)	0.137
SIRI a	0.21 (0.36)	0.19 (0.26)	0.053
C-reactive protein, (mg/L) a	1.16 (1.33)	1.15 (1.35)	0.132
Interleukin 6, (pg/ml) a	57.34 (45.02)	47.31 (56.11)	0.094

[a] median (interquartile range); *P <0.05 was considered statically significant.

Table 3. Demographic characteristics and clinical outcomes in twins

Characteristics	First twin (n=100)	Second twin (n=100)	P value
Gestational age, weeks [a]	28.1±1.1	28.1±1.1	1.000
Birth weight, g [a]	1130±189	1090±215	0.158
Antenatal steroid, n (%)	64 (64.0)	64 (64.0)	1.000
Male gender, n (%)	53 (53.0)	52 (52.0)	0.888
Cesarean section, n (%)	96 (96.0)	96 (96.0)	1.000
Chorioamnionitis, n (%)	16 (16.0)	16 (16.0)	1.000
Apgar 1st min, [b]	5 (2)	5 (2)	0.227
Apgar 5th min, [b]	7 (1)	7 (2)	0.309
Duration of MV, days [b]	1 (3)	1 (4)	0.978
EOS, n (%)	1 (1.0)	1 (1.0)	0.100
LOS, n (%)	14 (14.0)	22 (22.0)	0.199
RDS, n (%)	71 (71.0)	78 (78.0)	0.067
IVH, n (%)	12 (12.0)	13 (13.0)	0.178
PDA, n (%)	33 (33.0)	41 (41.0)	0.243
NEC, n (%)	2 (2.0)	2 (2.0)	1.000
BPD, n (%)	17 (17.0)	18 (18.0)	0.823
ROP, n (%)	10 (10.0)	12 (12.0)	0.434
Duration of hospitalization, days, [b]	53 (28)	56 (31)	0.646
Mortality, n (%)	10 (10.0)	11 (11.0)	0.819

[a] mean ± standard deviation, [b] median (interquartile range).

Table 4. Systemic inflammatory indices in twins

Parameters	First twin (n=100)	Second twin (n=100)	P value
Leukocyte count (103 μ/L) a	9.85 (8.59)	8.75 (6.23)	0.280
Platelet count (103 μ/L) a	240.00 (124.00)	216.00 (129.00)	0.223
Neutrophil count (103 μ/L) a	2.09 (2.08)	2.13 (1.70)	0.952
Monocyte count (103 μ/L) a	0.68 (0.45)	0.52 (0.42)	0.100
Lymphocyte count (103 μ/L) a	7.01 (5.58)	5.76 (5.01)	0.214
NLR a	0.28 (0.36)	0.33 (0.26)	0.055
MLR a	0.09 (0.05)	0.09 (0.04)	0.781
PLR a	40.01 (31.10)	42.61 (41.00)	0.126
PIV a	50.01 (51.77)	42.55 (52.85)	0.917
SII a	82.81 (75.52)	81.628 (75.19)	0.124
SIRI a	0.22 (0.28)	0.18 (0.23)	0.612
C-reactive protein, (mg/L) a	1.13 (1.30)	1.16 (1.38)	0.144
Interleukin 6, (pg/ml) a	44.07 (51.27)	49.62 (63.09)	0.102

a median (interquartile range).

Receiver-operating characteristic analysis was performed for systemic inflammatory indices that were statistically significant in terms of RDS and IVH between twins and singletons. The AUC value of PLR was 0.738 and the cut-off level was >36.1 for the prediction of RDS (p=0.0001). The AUC value of PLR was 0.701 and the cut-off level was >36.4 for the prediction of IVH (p=0.0001). The AUC value of PIV was 0.644 and the cut-off level was <44.2 for the prediction of RDS (p=0.0012). The AUC value of PIV was 0.602 and the cut-off level was <45.3 for the prediction of IVH (p=0.0022).

BPD, bronchopulmonary dysplasia; EOS, early onset sepsis; IVH, intraventricular hemorrhage; LOS, late onset sepsis; MV, mechanical ventilation; NEC, necrotising enterocolitis; PDA, patent ductus arteriosus; RDS, respiratory distress syndrome; ROP, retinopathy of prematurity.

MLR, monocyte to lymphocyte ratio; NLR, neutrophil to lymphocyte ratio; PIV, pan immune inflammation value; PLR, platelet to lymphocyte ratio; SII, systemic immune inflammation index; SIRI, systemic inflammation response index.

GA, BW, administration of antenatal steroid, male gender, cesarean section, chorioamnionitis, Apgar scores at 1st and 5th minutes, duration of MV support, EOS, LOS, RDS, IVH, PDA, NEC, BPD, ROP, duration of hospitalization, and mortality were similar between the first twin (n=100) and the second twin (n=100) preterm infants (p>0.05) (Table 3). The results of leukocyte, neutrophil, monocyte, lymphocyte, platelet count, CRP, IL-6, NLR, MLR, PLR, PIV, SII and SIRI values were similar between the first and the second twin infants (p>0.05) (Table 4).

DISCUSSION

Our study was the first to compared systemic inflammatory indices between singleton and twins and twins themselves in premature infants <32 weeks of gestation. Cesarean section, RDS, and IVH were higher in the twins group than the singleton group. Other demographical features and clinical outcomes, preterm morbidities were similar between the twins and singleton groups. Leukocyte, neutrophil, monocyte, lymphocyte count was lower and platelet count was higher in the twins group compared to singleton group. While systemic inflammatory indices including NLR, MLR, SII, and SIRI values were similar between the singleton and twin groups, PLR value was higher and PIV value was lower in the twins group compared to singleton group. According to the subgroup analysis, demographical features and clinical outcomes, preterm morbidities, leukocyte, neutrophil, monocyte, lymphocyte, platelet count and systemic inflammatory indices were found to be similar between the first and the second twins.

It has stated that maternal hematological values measured in pregnant women change as the week of pregnancy increases, and these values may differ from each other in singleton and twin pregnancies[17]. In a previous study, it was found that maternal NLR value was higher in twin pregnancies compared to singleton pregnancies in the first trimester, and maternal platelet value was lower in twin pregnancies in the second trimester compared to singleton pregnancies. In addition, it was reported that the results were similar between the twins and singleton groups in terms of maternal PLR[18].

While leukocyte, neutrophil, and monocyte counts are higher in term infants compared to late preterm infants, no difference was found between lymphocyte and platelet counts[19]. Christensen et al. have reported that neonatal mean eosinophil and monocyte values increase linearly as the gestational week increases[20]. On the contrary, Lee et al. have reported that there was no difference in the number of leukocytes, neutrophils, lymphocytes, monocytes, eosinophils and basophils in the umbilical cord of healthy newborns between different gestational weeks[21]. In fact, as the gestational weeks of newborns change, the distribution of hemogram parameters also changes normally. The predominant leukocyte is lymphocyte and monocytes in infants <32 weeks of GA. As the gestational week progresses, the number of lymphocytes and monocytes decreases, while the number of neutrophils increases. Moreover, it is known that the fetal platelet count increases normally in the later weeks of pregnancy[22,23]. Therefore, systemic inflammatory indices are likely to change dynamically with GA in premature infants. However, the effect of being twins on systemic inflammatory indices is unknown.

Monochorionic twins have a greater risk of serious complications than both dichorionic twins and singletons. After the occurrence of TTTS, differences occur between twins in terms of hemoglobin and platelet levels. However, it is not certain whether Leukocyte, neutrophil, and lymphocytes count will be affected[23]. There is less information on whether there is a difference in leukocyte parameters between twins without TTTS and among singleton premature infants. Sheffer-Sheffer-Mimouni et al. have reported that lymphocyte counts were higher in twins when comparing 74 concordant twins and 29 singleton control term infants. It is noteworthy that twin infants were more premature in this study. Higher lymphocyte counts may be associated with prematurity[25].

Shah et al. have reported that leukocyte, neutrophil and monocyte counts in infants with an average of 32 weeks of gestation were lower in multiple pregnancies than in singleton infants. Furthermore, they have not found any difference between the groups in terms of platelet count[2]. In our study, leukocyte, neutrophil, monocyte, and lymphocyte count was low and platelet count was high in the twins group. This may be explained that even if they have the same gestational week, twin premature infants may respond in the form of lower leukocyte, neutrophil, monocyte, lymphocyte count and higher platelet count as they are in a relatively hypoxic environment compared to singletons. In previous studies, the authors explain the evidence that twin infants are

more hypoxic with a higher nucleated red blood cell count[2]. However, the nucleated red blood cell count could not be evaluated in our study.

Most of the information on hematological parameters comes from small studies, and the results of these studies are limited to guide because of the lack of comprehensive information. Additionally, it is still unclear how all these leukocyte parameters are reflected in the clinical settings[2,20,22-25]. Therefore, more information about systemic inflammatory indices is needed to enable the clinical use of parameters derived from CBC. It has been reported that systemic inflammatory indices can be prominent parameters that can be used to determine the prognosis of neurological, oncological, respiratory, cardiovascular and infectious diseases in the adult and pediatric diseases[4,5,26-30]. In recent years, it has been stated that systemic inflammatory indices may be helpful parameters in the diagnosis of some diseases such as HIE, sepsis, ROP, IVH, NEC, RDS, BPD, and PDA in the field of neonatology[6-9,31-34]. However, it is not known whether these systemic inflammatory indices, which are still new in the field of neonatology, vary normally between twin infants and compared to singleton infants.

Furthermore, the effect on the clinical outcomes of systemic inflammatory indices, which varies by being twins, is unknown. In our study, the PLR value was higher and the PIV value was lower in the twins group compared to the singleton group. NLR, MLR, SII, and SIRI values were similar between singleton and twin groups. While GA and BW were similar between twins and singleton groups, the frequency of RDS and IVH was higher in twin infants. Stein et al. reported that high PLR value was associated with seizure in 13 preterm infants with IVH. However, no comparison was made with a control group without IVH in this study[9]. No information is existed yet on whether there is a relationship between RDS and systemic inflammatory indices. However, based on our results, we can hypothesize that high PLR value and low PIV value may be associated with RDS and IVH. Since the aim of our study was whether being a twin infant affects systemic inflammatory indices, the relationship between clinical outcomes and systemic inflammatory indices was not evaluated. Our study may shed light on the evaluation of the morbidities of the twin preterms and the relationship of systemic inflammatory indices in the future studies. In our study, no difference was found between the first and the second twin infants in terms of demographic features, morbidities, mortality, leukocyte, neutrophil, monocyte, lymphocyte, platelet count and systemic inflammatory indices. Thus, it can be concluded that both leukocyte, neutrophil, monocyte, lymphocyte, platelet count and systemic inflammatory indices were not affected due to the lack of discordance in our patients[1]. In the future studies, it can be evaluated whether systemic inflammatory indices are affected in twins with and without discordance and their impact on clinical outcomes.

The main limitation of our study was that it was conducted from a single center and its retrospective design. Our results were interpreted according to the complete blood count results obtained in the first hour after birth. Serial follow-up of systemic inflammatory indices in postnatal days could not be performed and the values in term infants could not be compared. Additionally, maternal complete blood count results, maternal diseases and drugs, placental pathological examination could not be performed.

In conclusion, our study was the first to evaluate the effect of premature twins on systemic inflammatory indices. There was no difference in systemic inflammatory indices between twin premature infants. However, PLR value was higher and PIV value was lower in twin preterms compared to singleton ones. The higher frequency of RDS and IVH we detected in twin preterms may be related to high PLR value and low PIV value. Prospective studies evaluating systemic inflammatory indices and morbidity of prematurity in twin preterms are needed to test and substantiate this finding.

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