Inflammatory Cell Response in Cases of Neonatal Encephalopathy Treated with Therapeutic Hypothermia

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Abstract

Background: Antenatal inflammation is associated with the increasingly severe and negative neurological findings of neonatal ecephalopathy. A reduction in antenatal inflammation reduces neurological damage. The effect of therapeutic hypothermia on inflammation is not clear and remains the subject of research. Aim: The aim of this study was to investigate the inflammatory cell response in neonatal ecephalopathy cases treated with therapeutic hypothermia. Methods: The study included a total of 102 cases, 51 cases diagnosed with perinatal asphyxia and a control group of 51 healthy newborns. Blood samples were taken before therapeutic hypothermia treatment and at the 24th and 72th hours of treatment in patients with perinatal asphyxia. In the control group, blood samples were taken in the first 6 hours postnatally. Results: In the asphytic group, mean leukocyte ($p<0.001$), neutrophil ($p<0.001$), and lymphocyte ($p=0.\ 014$) values within the first 6 hours were significantly higher than those of the control group. The specificity for leukocyte, neutrophil and lymphocyte (measured before TH) was 80.4%, 88.2% and 60.8%, and sensitivity was 84.3%, 88.2% and 62.7%, respectively. The mean leukocyte, thrombocyte, and neutrophil values during the first 6 hours after delivery were significantly higher than the mean values at the 24th and 72nd hours after TH ($p<0.001$), and the mean platelet volume values were significantly lower ($p<0.001$). Conclusion: High leukocyte, neutrophil and lymphocyte values and low thrombocyte count in the first 6 hours of life may be an early sign of perinatal asphyxia and can be used as a marker to start treatment. It is thought that by decreasing the number of inflammatory cells, therapeutic hypothermia reduces the severity of encephalopathy and potentially negative results.

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Abstract

Background: Antenatal inflammation is associated with the increasingly severe and negative neurological findings of neonatal ecephalopathy (NE). A reduction in antenatal inflammation reduces neurological damage. The effect of therapeutic hypothermia (TH) on inflammation is not clear and remains the subject of research.

Aim: The aim of this study was to investigate the inflammatory cell response in NE cases treated with therapeutic hypothermia (TH).

Methods: The study included a total of 102 cases, 51 cases diagnosed with perinatal asphyxia and a control group of 51 healthy newborns. Blood samples were taken before TH treatment and at the 24th and 72th hours of treatment in patients with perinatal asphyxia. In the control group, blood samples were taken in the first 6 hours postnatally.

Results: In the asphytic group, mean leukocyte ($p<0.001$), neutrophil ($p<0.001$), and lymphocyte ($p=0.014$) values within the first 6 hours were significantly higher than those of the control group. The specificity for leukocyte, neutrophil and lymphocyte (measured before TH) was 80.4%, 88.2% and 60.8%, and sensitivity was 84.3%, 88.2% and 62.7%, respectively. The mean leukocyte, thrombocyte, and neutrophil values during the first 6 hours after delivery were significantly higher than the mean values at the 24th and 72nd hours after TH ($p<0.001$), and the mean platelet volume values were significantly lower ($p<0.001$).

Conclusion: High leukocyte, neutrophil and lymphocyte values and low thrombocyte count in the first 6 hours of life may be an early sign of perinatal asphyxia and can be used as a marker to start treatment. It is thought that by decreasing the number of inflammatory cells, therapeutic hypothermia reduces the severity of encephalopathy and potentially negative results.

What's known:

Brain damage is the most significant cause of neurological loss in term infants and birth asphyxia is responsible for most of these issues.

Hypoxia-ischemia-reperfusion injury in the central nervous system activates the chain of pro-inflammatory events and initiates leukocyte flow into this area.

What's new:

Mean white blood cells, neutrophils and lymphocyte values were significantly higher in the perinatal asphyxia group than in the healthy control group during in the first six hours of life.

High white blood cells, lymphocyte, and neutrophil values can be indicators of asphyxia during the first 6 hours in perinatal asphyxia cases.

Key words: Newborn, neonatal encephalopathy, neutrophil, inflammatory cell, therapeutic hypothermia

Introduction

Hypoxic-ischemic encephalopathy is a major cause of infant mortality and morbidity with long-term neurological sequelae. Hypoxic brain injury is an evolving process that results from an initial insult and extends during the reperfusion phase of injury.[1,2] It is characterized by symptoms such as seizure, difficulty in starting and maintaining respiration, circulatory impairment, abnormal muscular tonus, and a decrease in reflexes.[3]

Transient ischemia of the cerebral vasculature followed by reperfusion leads to a secondary cascade of pathophysiological events, characterized by a complex inflammatory response.[4] In particular, it activates immune cells in hypoxia-ischemia-induced cell damage or cell death, microglia, and astrocytes. These activated cells lead to the synthesis and production of pro-inflammatory cytokines such as interleukin 1b, interleukin 6, and tumor necrosis factor-a. Pro-inflammatory cytokines increase the expression of mediators that induce vascular permeability, the migration of leukocytes, and promote local inflammatory reactions.[5-7] The production of leukocytes and the number of circulating neutrophils increase. Cell damage occurs due to the production of reactive and toxic metabolites such as hydrogen peroxide, superoxide anion, and hypochlorous acid from neutrophils.[8] Thrombocytes play a fundamental role in hemostasis. However, recent studies have revealed that thrombocytes also play an essential role in infection and inflammation.[9,10] Mean platelet volume (MPV) is a value that indicates thrombocyte activation and can be used as a biomarker in inflammation. Changes in the MPV have been studied in many diseases.[11-13] Therapeutic hypothermia constitutes one of the most important therapies providing neuroprotection, and has been reported to reduce brain damage by reducing the production of inflammatory cells and inflammatory cytokines.[14]

The aim of this study was to investigate the inflammatory cell response in NE before and during TH.

Methods

This prospective study included a total of 102 cases, comprising 51 infants admitted to a tertiary level Neonatal Intensive Care Unit with a diagnosis of NE and treated with hypothermia between January 2019 and June 2020, and a control group of 51 healthy newborns. Before the study, written informed consent was obtained from the parents of the patients who participated in the research. Approval for the study was granted by the Ethics Committee of Harran University Medical Faculty (Approval date: 07.01.2019, Session 1, Number: 19.01.03). All procedures were applied in compliance with the Helsinki Declaration.

Inclusion Criteria: All cases included in the perinatal asphyxia group were admitted to our unit for the application of therapeutic hypothermia treatment. Therapeutic hypothermia was administered to patients born at [?]36 weeks, aged [?]6 hours, had cord blood or blood gas taken within the first hour after birth showing a pH value of [?]7.00 and/or with base deficit [?]16 mmol/L, had a 10-minute Apgar score of <5, had an ongoing need for resuscitation and showed signs of medium or heavy encephalopathy in the clinical evaluation. In both the clinical assessment and the follow-up period, aEEG findings were also taken into account.[15,16]

Exclusion Criteria: Infants were excluded from the study if they were aged > 6 hours, were born before the 36^{th} week of pregnancy, weighed under 2000 grams, had an uncertain diagnosis or the presence of other documented conditions which could cause neonatal encephalopathy, congenital metabolic diseases, a diagnosed energy deficiency in the family with a sibling's medical history and other accompanying diseases together with early encephalopathy, who were thought to be unlikely to benefit from the treatment, had severe or extensive parenchymal cranial hemorrhages, severe life-threatening coagulopathy, maternal chorioamnionitis, trisomy's, multiple organ anomalies, bacterial growth in blood cultures taken within the first 72 hours of life and/or were suspected of having early-onset neonatal sepsis.[15-16]

All patients included in the perinatal asphyxia group received TH treatment. A total of 51 patients underwent TH with the whole body cooling system. Therapeutic hypothermia was applied using the Arctic Sun® 5000 Temperature Management System as servo-controlled whole body cooling with a rectal temperature probe targeting a rectal temperature of 33.5°C. After 72 hours of cooling, 7 hours of re-warming was applied (maximum temperature rise of 0.5 ° C / hour) and the session was finished when the body temperature reached 36.5°C.

In the control group, blood samples were taken within the first 0-6 hours after birth from healthy, term infants who were given to their mothers following the birth and whose physical examination showed no pathological findings. In addition, there were no pathological findings during pregnancy of the mothers of these babies.

Blood Withdrawal and Analysis: Blood samples of 2mL were taken from all the cases studied for 0-6 hours postpartum and from cases with hypothermia diagnosed with NE only at 24 and 72 hours. Whole blood count analysis was performed on the samples obtained using an automated blood count device (Abbot Celldyn 3500 Ill, USA). As a result of the complete blood count analysis, leukocyte, neutrophil, lymphocyte, thrombocyte and MPV values were obtained.

Data Analysis: Statistical analyses were performed using SPSS version 24.0 software (SPSS Inc, Chicago, IL, USA). Descriptive statistics were summarized as number, percentage, mean, and standard deviation values. Normal distribution of the variables was checked visually (histogram and probability charts) and using analytical methods (Kolmogorov-Smirnov test). Data showing normal distribution were analyzed using the Independent Samples t-test. The analysis of skewed variables was performed using the Mann Whitney U-test. The repeat measurements of the perinatal asphyxia groups were compared with the Paired Samples t-test. Categorical variables were analyzed using the Pearson Chi-square test. Specificity and sensitivity analysis were performed by using receiver operating characteristic (ROC) curve analysis technique. In the ROC analysis, the area under the curve (AUC) values were studied. A value of $p<0.05$ was accepted as statistically significant.

Results

Evaluation was made of a total of 102 children, comprising 51 (50%) in the NE group (51% males, 49% females) and 51 (50%) in the control group (54.9% males, 45.1% females). No significant difference was found between the two groups in terms of gender distribution. The average weight of the cases was 3255.7±417.98 gr in the NE group and 3318.8±369.25 gr in the control group. No significant difference was determined between the two groups in respect of birth weight, and the birth week distribution was similar in both groups (Table 1).

The laboratory test results of the NE group and control group were compared. The mean leukocyte and neutrophil values were significantly higher in the NE group, and there was no difference in the mean platelet values. There was no significant difference between the NE group and the control group in terms of the median MPV value, and the median lymphocyte value was significantly higher in the NE group than in the control group (Table 2).

ROC curves were plotted to compare the predictive values of leukocyte, neutrophil and lymphocyte in all 102 patients. As a result of the analysis, for the leukocyte value of [?]14.99 10e3/uL, the area under the curve (AUC) was determined to be 0.908 ($p<0.001$) with 84.3% sensitivity and 80.4% specificity. For the neutrophil value of [?]8.18 10e3/uL, AUC: 0.921 ($p<0.001$) was determined with 88.2% sensitivity and 88.2% specificity. For the lymphocyte value of [?] 4.91 10e3/uL, AUC: 0.641, ($p=0.022$) was determined with 62.7% sensitivity and 60.8% specificity. The ROC analysis showed no statistical significance for MPV (AUC: 0404, $p = 0.094$) and thrombocyte (AUC: 0.424, $p = 0.184$) (Figure 1).

The changes in the results of the blood count analysis of the cases in the NE group during the TH process were analyzed. As a result of the analysis, a significant difference was observed in the leukocyte, neutrophil, MPV and platelet measurements taken in the first 6 hours of life from the values measured after 24 and 72 hours of TH treatment. No significant difference was found in lymphocyte measurements (Table 3).

The leukocyte and neutrophil levels were determined to be high before TH treatment, and significantly decreased at 24 and 72 hours of TH treatment. In the first 24 hours of TH treatment, it was found that the platelet count decreased and MPV levels increased, and there was no significant change in the platelet count and MPV levels during the continuation of the treatment (Table 4).

Discussion

Severe and prolonged ischemia or hypoxia of any organ causes cell death and tissue damage. The extent of the cerebral injury after the hypoxic-ischemic event, the mechanism of the irreversible damage such as neuronal necrosis or permanent inflammation, and acute phase response, depend on the recovery and the neuronal repair balance. Hypoxia-ischemia-reperfusion injury has been reported to cause a pro-inflammatory

chain of events.[9,17] In cases of brain damage caused by asphyxia, both microvascular damage and cell injury are vital factors.[18] In a study by Wassink et al.[19] it was concluded that the hypoxia-ischemia-reperfusion damage in the central nervous system activates a chain of events characterized by leukocyte flow, including polymorphous nuclear cells and monocytes and microglial activation. In an experimental animal study by Hudome S. et al.[20] it was reported that neutrophils and lymphocytes increased in the period following ischaemic injury and this increase is usually related to the severity of the injury and long-term neurological results. Shah V. et al.[21] reported that despite no correlation between lymphocyte count and outcome, high peripheral neutrophil count was related to worse neurological outcomes. Jenkins DD et al.[22] determined a significant increase in leukocyte, neutrophil and lymphocyte levels examined at 0-9 hours in NE cases. In the current study, the first 6-hour laboratory results of the NE and control groups were compared. Mean leukocyte, neutrophil and lymphocyte values were found to be significantly higher in the NE group than in the control group. The leukocyte increase was thought to be due to polymorphonuclear cell activation secondary to hypoxia-ischemia-reperfusion injury.

Therapeutic hypothermia, or TBC, is a treatment method with proven beneficial effects on the outcomes of moderate and severe NE. Low leukocyte levels in the circulation following NE in a newborn have been associated with hypothermia treatment. A greater decrease in leukocyte, neutrophil, and lymphocyte counts has been observed in cases applied with hypothermia treatment. The possible mechanisms for the hypothermia effect include bone marrow suppression and decreased leukocyte expression.[23,24] Jenkins DD et al.[22]compared NE cases applied and not applied with hypothermia treatment and determined that the leukocyte count in the hypothermia treatment group showed a more rapid decrease and reached a significantly lower level within 24 hours compared to the normothermic group. Hypothermia treatment was reported to be related to significantly lower leukocyte and neutrophil values compared to the normothermic cases. Furthermore, the lymphocyte count in the hypothermia group was significantly lower after 36 hours compared to the normothermia group. In the current study, laboratory analyses performed at 0-6, 24 and 72 hours after birth were compared. The mean leukocyte and neutrophil values of the NE group cases measured during therapeutic hypothermia treatment at 24 and 72 hours postpartum showed a significant decrease compared to those measured during the first 0-6 hours of life without therapeutic hypothermia treatment. However, lymphocyte values did not change significantly.

Mean platelet volume is an indicator of thrombocyte function and activation. Large thrombocytes are hemostatic and more active. As young thrombocytes are larger than older ones, thrombocyte counts and MPV are inversely related. MPV appears to be an indicator of thrombocyte production and consumption, in addition to some serious diseases related to hypoxia, perinatal infections, and bone marrow.[25,26] In a study of 106 patients stained with meconium amniotic fluid, Tekin et al.[27] found the MPV level during the first 2 hours of life to be significantly lower than that of the control group. However, no significant difference was observed in the thrombocyte levels. The low level of MPV was considered to be secondary to hypoxia.[27] In a study of 55 cases diagnosed with perinatal asphyxia, Kannar et al. found that the MPV level during the first 24 hours after birth was significantly higher than that of the control group, while the thrombocyte level was considerably lower.[28] In the current study, mean thrombocyte and MPV values in the first 6 hours did not differ significantly from those of the control group. The mean thrombocyte values of the NE group at 24 and 72 hours showed a significant decrease compared to the first 6 hours, while the mean MPV levels increased significantly.

Conclusion

The results of this study have revealed that high leukocyte, lymphocyte and neutrophil values can be indicators of asphyxia during the first 6 hours in NE cases and can therefore be used as a marker to initiate early treatment. The mean leukocyte, neutrophil and thrombocyte values of the NE group at 24 and 72 hours decreased significantly compared to the first 6 hours and the increase in MPV was thought to be due to the immunosuppression of the hypothermic treatment and bone marrow suppression.

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Ethics approval: Approval was obtained from the ethics committee of Harran University. The procedures used in this study adhere to the tenets of the Declaration of Helsinki.

Conflict of interest: The authors declare that they have no conflict of interest.

Consent to participate: Informed consent was obtained from all individual participants included in the study.

Authors' contributions: HG: Idea and study design, literature research, data collection, data analysis, manuscript writing, HK: Data collection, manuscript writing, DK: Case referral, interpretation of findings, AS: Study design, interpretation of findings.

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Table 1.Sociodemographic characteristics of perinatal asphyxia and control group cases

	PA (n=51)	**Control (n=51)**	P value
Gender M/F, (%)	51/49	54.9/45.1	[a]0.69
Birthweek, (n/%) 36. 37. 38. 39. 40.	4/7.8 12/23.5 23/45.1 5/9.8 7/13.7	5/9.8 9/17.6 19/37.3 6/11.8 12/23.5	[a]0.67
Birthweight, gr(mean±SD)	3255.7±417.98	3318.8±369.25	[b]0.42
PA: Perinatal asphyxia; SD: Standard deviation; [a]: Fisher's Exact test; [b]: Independent Samples t-test	PA: Perinatal asphyxia; SD: Standard deviation; [a]: Fisher's Exact test; [b]: Independent Samples t-test	PA: Perinatal asphyxia; SD: Standard deviation; [a]: Fisher's Exact test; [b]: Independent Samples t-test	PA: Perinatal asphyxia; SD: Standard deviation; [a]: Fisher's Exact test; [b]: Independent Samples t-test

Table 2.Comparison of laboratory analysis results of the perinatal asphyxia and control groups within the first 6 hours.

Hematology (Unit)	**PA (n=51)**	**Control (n=51)**	P value
WBC (10e3/uL) Mean±SD	21.95±7.05	12.81±2.67	[a]<0.001
NEU (10e3/uL) Mean±SD	13.34±5.43	6.09±1.57	[a]<0.001
LYM (10e3/uL) Median(min-max)	5.5(2.3-16.95)	4.68(2.28-9.95)	[b]0.014
MPV (fL) Median(min-max)	6.06(4.88-12.29)	6.39(4.59-9.09)	[b]0.094

Hematology (Unit)	PA (n=51)	Control (n=51)	P value
PLT (10e3/uL) Mean±SD	244.73±90.76	268.09±66.17	[a]0.14
SD: Standard deviation; WBC: white blood cell; PLT: platelet; NEU: neutrophil; LYM: lymphocyte; MPV: mean platelet volume PA: perinatal asphyxia; SD: standard deviation; [a]: independent Samples t-test; [b]: Mann Whitney U-test	SD: Standard deviation; WBC: white blood cell; PLT: platelet; NEU: neutrophil; LYM: lymphocyte; MPV: mean platelet volume PA: perinatal asphyxia; SD: standard deviation; [a]: independent Samples t-test; [b]: Mann Whitney U-test	SD: Standard deviation; WBC: white blood cell; PLT: platelet; NEU: neutrophil; LYM: lymphocyte; MPV: mean platelet volume PA: perinatal asphyxia; SD: standard deviation; [a]: independent Samples t-test; [b]: Mann Whitney U-test	SD: Standard deviation; WBC: white blood cell; PLT: platelet; NEU: neutrophil; LYM: lymphocyte; MPV: mean platelet volume PA: perinatal asphyxia; SD: standard deviation; [a]: independent Samples t-test; [b]: Mann Whitney U-test

Table 3.Comparison of blood samples taken in the first 6 hours of life and at 24 and 72 hours

Perinatal asphyxia group	1.	2.	3.	[b]*P value*
WBC (10e3/uL) Median (min-max)	20.9(10-48.1)	15.3(6.4-39.2)	13.3(3.3-55.4)	<0.001
NEU (10e3/uL) Median (min-max)	12.6(2.38-29)	7.5(2.4-24.30)	5.20(1.6-15)	<0.001
LYM (10e3/uL) Median (min-max)	5.5(2.3-16.95)	4.8(0.8-22.6)	6.4(1.5-50)	0.156
MPV (fL) Median (min-max)	6.06(4.88-12.29)	6.12(5.50-10.8)	6.1(5.3-13)	<0.001
PLT (10e3/uL) Median (min-max)	249.9(45.86-487)	196(54-433)	212(22-691)	<0.001

1. : The first 6 hours of life 2. :24 hours of life 3. :72 hours of life; WBC: White blood cell; PLT: platelet; NEU: Neutrophil; LYM: Lymphocyte; MPV: Mean platelet volume; PA: Perinatal asphyxia;[a]: Friedman test sig.

Table 4.Comparison of blood samples taken in the first 6 hours of life and at 24 and 72 hours

PA cases (n=51)
WBC
NEU
MPV
PLT
1. : The first 6 hours of life 2. :24 hours of life 3. :72 hours of life; WBC: White blood cell; PLT:platelet; NEU: Neutrophil;

Figure1. ROC curve graph for white blood cell(WBC), neutrophil (NEU), lymphocyte (LYM), mean platelet volume (MPV) and platelet (PLT) values