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Original Article

An investigation of the relationship between base deficit and CRP in asphytic infants

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Abstract

Aim: To investigate the relationship between base deficit (BD) and C-reactive protein (CRP) findings evaluated during diagnosis of infants followed up for a diagnosis of perinatal asphyxia (PA).

Material and Methods: This prospective observational study included 66 cases as 33 cases treated and followed up for a diagnosis of PA in the Neonatal Intensive Care Unit of Harran University Medical Faculty Hospital, and a control group of 33 healthy infants. Using a syringe washed with heparin, a fetal blood sample of 2ml was taken from the umbilical artery of the neonates thought to have PA, and blood gases were examined in an anaerobic environment. CRP values were obtained from spectrophotometric biochemical analysis of the 2ml blood sample using an Architect C16000 device (Abbott Diagnostics, Abbott Park, IL, USA).

Results: Evaluation was made of 33 infants with a diagnosis of PA hospitalised in NICU and a control group of 33 healthy infants. All the cases were statistically similar in respect of gestational week at birth (GW), birthweight (BW), and gender. In the blood gas examinations of the cord blood, pH value was determined as mean 6.9 (range, 6.50-7.06), mean BD as 17mmol/L (range, 12-28 mmol/L), and CRP values as 0.2-57 mg/dl. The CRP values of the PA cases on days 2 and 3 were determined to be significantly higher than those of the control group ($p<0.001$). As the BD value at the time of diagnosis increased, so there was determined to be a significant increase in CRP value, and this was confirmed by the Spearman correlation test (r1:0.423, p1:0.014) (r2:0.342, p2:0.05) (r3:0.451, p3:0.009). No statistically significant relationship was determined between the pH value in blood gas and the CRP level (p:0.18).

Conclusion: The results of this study demonstrated that the CRP level was statistically significantly higher in cases that developed asphyxia than in the control group. A positive correlation was determined between BD at diagnosis and CRP level. A high CRP level during treatment and follow-up can be considered for use as a supportive finding showing the severity of hypoxia.

Keywords: Asphyxia, base deficit, C-reactive protein

INTRODUCTION

Perinatal asphyxia (PA) is the most significant cause of neurological morbidity which can develop later in term and premature infants (1).

Anaerobic metabolism secondary to hypoxia is used to be able to maintain normal functions in the neonatal brain. Anaerobic metabolism leads to an increase in the level of lactic acid, disruption of normal metabolic activity and metabolic acidosis (2). pH and BD values show metabolic acidosis. In the blood gases examined from the fetal cord, a pH value of <7.00 mmol/L suggests significant fetal acidemia, BD of 12-16 mmol/L that the infant had a hypoxic birth and BD >16 mmol/L that the infant remained in severe hypoxia (3). Increased BD (> 20-25) is associated with poor prognosis (4). As the severity of asphyxia in the infant increases, so the prognosis worsens and morbidity and mortality rates increase (5).

Severe and long-term ischaemia or hypoxia of any organ causes cell death and tissue damage. In addition to infection, CRP in newborns can be elevated in non-infectious conditions (asphyxia, respiratory distress syndrome, intracranial hemorrhage, meconium aspiration pneumonia, and pre-eclampsia) which cause inflammation or tissue damage. CRP does not pass from the placenta or passes at a minimal level. Therefore, CRP elevation in the fetus or newborn is not of maternal origin. CRP is not affected by the degree of prematurity or gestational age (6).

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The aim of this study was to investigate the relationship between BD, which shows hypoxic birth, and CRP level, in infants followed up for a diagnosis of PA, through evaluation of BD and CRP findings at the time of diagnosis.

MATERIAL and METHODS

The study included a total of 66 cases, comprising 33 infants admitted to the tertiary level Neonatal Intensive Care Unit (NICU) with a diagnosis of PA and treated with hypothermia between January and December 2019, and a control group of 33 healthy newborns. Before the study, written informed consent was obtained from the parents of the patients who participated in this study. This study conformed to the principles of the 2008 Declaration of Helsinki and was approved by the ethics committee of Harran University School of Medicine (Approval date: 07.01.2019, Session 1, Number: 19.01.03). The patients included were those with gestational age ≥ 36 weeks, and pH ≤7.00 or BD ≥ 16mmol/L in cord blood gas at ≤6 hours or within the first hour postnatal, a 10-min Apgar score <5 or a continued need for resuscitation, and findings of moderate or severe encephalopathy in clinical evaluation. In addition to these criteria, both diagnostic and follow-up aEEG findings were added (7,8).

Exclusion criteria were defined as birthweight <2000 gr, those with congenital metabolic disease, sibling history, other diseases seen with energy deficiency and early encephalopathy, severe or widespread cranial parenchymal bleeding, life-threatening coagulopathy, a history of maternal chorioamnionitis, blood-culture positivity, trisomy, or multiple organ anomalies (7, 8).

A total of 33 patients had undergone therapeutic hypothermia. Patients underwent therapeutic hypothermia with whole body cooling system. Therapeutic hypothermia was applied with 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 period, 7 hours of rewarming period occurred (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 obtained from infants who were given to their mothers after birth and whose physical examination was normal.

Blood Sampling and Analyses

Using a syringe washed with heparin, a 2cc fetal blood sample was taken from the umbilical artery for blood gas examination in an anaerobic environment. After taking the sample, the needle tip was bent and covered with a plastic cover, preventing oxygen from coming into contact with the fetal blood. The blood gas parameters were examined within the first 30 minutes in cold chain conditions. Blood samples are taken from PA patients on the 1st, 2nd, 3rd, 4th, and 7th day to assess CRP levels and blood cultures are obtained from all of the PA patients on admission to NICU and when CRP levels were found to be elevated. Blood samples were taken from healthy participants in the control group between 48-72 hours. The CRP values were obtained using the Architect C16000 spectrophotometric chemical analysis device (Abbott Diagnostics, Abbott Park, USA).

Statistical Analyses

Data obtained in the study were analysed statistically using SPSS vn 24.0 software (SPSS Inc. Chicago, IL, USA). Descriptive statistics were summarised as number, percentage, mean and standard deviation (SD) values. Conformity of the data to normal distribution was assessed with visual (histogram, probability tables) and analytical (Kolmogorov-Smirnov) methods. Analysis of data with normal distribution was made with the Independent Samples t-test, and the Mann Whitney U-test was applied to data not showing normal distribution. In the comparison of qualitative data between groups, the Chi-square test was applied. For the comparisons of repeated continuous measurements, Friedman analysis was applied to non-parametric data and variance analysis to parametric data. CRP variations during hospitalisation were evaluated with the Wilcoxon Signed Rank test. To analyse the relationships between variables, Pearson correlation was used for parametric variables and Spearman correlation for non-parametric variables. A value of $p<0.05$ was accepted as statistically significant in all the analyses.

RESULTS

The study included 66 cases admitted to the NICU of a university medical faculty hospital, as 33 (50%) cases in the PA group and 33 (50%) in the control group. The PA group comprised 69.7% males and 30.3% females, and the control group, 54.5% males and 45.5% females. No significant difference was determined between the groups in respect of gender distribution. The mean gestional week was 38.75±1.32 weeks in the PA group and 38.24±1.09 weeks in the control group, with no significant difference determined between the groups. Birthweight was mean 3270.75±444.08 gr in the PA group and 3218.48±404.82 gr in the control group with no significant difference determined between the groups. In the PA group, CRP positivity was determined in 6.1% of patients on Day 1, in 39.4% on Day 2 and in 72.7% on Day 3. The change in CRP levels showed a pattern of increasing from the lowest level on Day 1 to the highest level on Day 3 ($p<0.0001$) (Figure 1, Table 1).

The CRP levels of the patients diagnosed with PA were similar to those of the control group on Day 1, and were higher on Days 2 and 3. The CRP levels of the PA patients showed a statistically significant change over time (Table 2).

In the cases diagnosed with PA, the pH value in the blood gas examination of the cord blood taken in the first hour postnatal was mean 6.87±0.12 (median 6.9, range 6.50-7.06). The BD value in the blood gas examination of the cord blood taken in the first hour postnatal was mean 18.18±4.31mmol/L (range, 12-28 mmol/L). A positive

correlation was determined between the mean BD at the time of diagnosis and the CRP values on Days 1, 2, and 3 (Table3).

No statistically significant relationship was determined between the pH value in the blood gas and CRP level (p:0.18).

Table 1. The change in CRP levels in the patients diagnosed with PA

	N	Minimum	Maximum	Median	p
C-CRP	33	0.2	5.3	0.76	
CRP1	33	.20	7.00	1.00	0.000
CRP2	33	.55	30.00	3.78	
CRP3	33	1.39	57.00	9.62	

C-CRP: control group, CRP1: CRP on Day 1, CRP2: CRP on Day 2, CRP3: CRP on Day 3 *Friedman Test

Table 2. Evaluation of the PA and Control groups in respect of CRP

	C.CRP- CRP1	C.CRP- CRP2	C.CRP- CRP3	CRP1- CRP2	CRP1- CRP3	CRP2- CRP3
Z	-.954	-4.315	-4.941	-4.816	-5.012	-4.994
P*	.340	.000	.000	.000	.000	.000

* Wilcoxon Signed Rank Test, C-reactive protein(CRP), C-CRP: control group, CRP1: CRP on Day 1, CRP2: CRP on Day 2, CRP3: CRP on Day 3

Table 3. Evaluation of the PA and Control groups in respect of CRP

	Min-Max(Md)	[b]r	[a]P
BD(mmol/L)	12-28(17)		
CRP1(mg/l)	0.2-7(1)	.423	0.014*
CRP2(mg/l)	0.55-30(3.8)	.342	0.05
CRP3(mg/l)	1.39-57(9.62)	.451	0.009**

C-CRP: control group, CRP1: CRP on Day 1, CRP2: CRP on Day 2, CRP3: CRP on Day 3, BD: Base Deficit, Min: Minimum, Max: Maximum, Md: Median, a,Spearman correlation test, b correlation coefficient, *. Correlation is significant at the level of 0.05, ** Correlation is significant at the level of 0.01

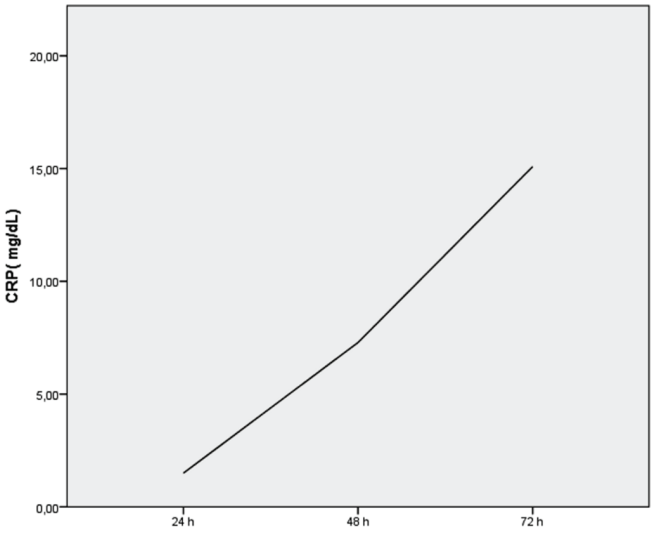

Figure 1. Change in CRP level during therapeutic hypothermia treatment

DISCUSSION

Perinatal asphyxia is a condition that results in the development of arterial hypoxemia, hypercarbia and acidosis, because of deterioration in pulmonary ventilation caused by insufficient gas exchange in the placenta or postnatal events (9,10). Hypothermia is the most effective treatment used to reduce morbidity and mortality in patients who develop asphyxia. The appropriate criteria for hypothermia treatment are pH≤7.0 or BD≥16 mmol/L in the blood sample or umbilical cord blood in the first postnatal hour, history of an acute perinatal event, a 10-min Apgar score <5, and neurological examination findings showing moderate to severe encephalopathy (11). The current study included patients diagnosed with PA and applied with hypothermia treatment. The pH values in the blood gas examined from the cord blood were in 6.87±0.12. The BD in the blood gas examined from the cord blood was in 18.18±4.31 mmol/L. Findings of moderate to severe encephalopathy were seen according

to the Sarnat criteria.

CRP is expressed by the liver in response to various inflammatory cytokines. Therefore, CRP measurement is widely used to monitor various inflammatory conditions (12). Inflammatory markers are commonly elevated in neonatal encephalopathy even without coexistent infection (13). In a study by Aibiki M et al. (14) they found that therapeutic hypothermia reduced inflammation. In a study by Chakkarapani E et al. (15) therapeutic hypothermia has been reported to delay CRP response in infants. The degree of cerebral injury following a hypoxic-ischaemic event and the repair mechanism of irreversible damage such as neuronal necrosis or permanant inflammation are associated with the balance of acute phase response, healing and neuronal repair (16). Bonestroo HJC et al (17) reported that hypoxia-ischaemia-reperfusion damage in the central nervous system (CNS) activated a chain of pro-inflammatory events. Wassink G et al (18) determined that most cytokines of inflammatory reactions in the CNS mediated neuronal apoptosis modulation in particular. In a study by Okumus et al. (19), 82.5% of therapeutic hypothermia patients had positive CRP on day 4 despite negative culture results. They found that the change in CRP level during therapeutic hypothermia was lowest on day 1 and highest on day 3. ın the current study, CRP positivity was determined in 6.1% of the PA patients on Day 1, in 39.4% on Day 2 and in 72.7% on Day 3. While the CRP values were similar to those of the control group on Day 1, despite the negative blood culture results and absence of signs of clinical septicemia, the CRP levels of the PA patients were higher after the first day. On Days 2 and 3, the CRP levels of the PA patients were statistically significantly higher than those of the control group ($p<0.001$). In a study by Okumus et al. (19) they thought that elevated CRP levels were due to therapeutic hypothermia treatment. However, Chakkarapani E et al. (15) found that therapeutic hypothermia suppressed inflammation and delayed CRP response in infants. Inflammatory markers are increasing in neonatal encephalopathy. CRP levels increase in response to increased inflammatory markers secondary to brain damage (12,13). We think that CRP levels increase in response to increased inflammatory markers secondary to brain injury. CRP levels gradually increased to reach the highest level on the third day of TH treatment. We think TH is due to reducing inflammation and delaying CRP response.

Hypoxic birth asphyxia is a serious condition with mortality rates as high as 35% (20-22). The inclusion of active respiration and neurological treatment in modern intensive care plays an important role in predicting the prognosis of asphytic infants and the decisions of whether to continue or withdraw care (23). In a study by Papile LA et al (24), it was reported that BD of 12-16 mmol/L indicates hypoxic birth of the infant, and >16mmol/L suggests that the infant remained in severe hypoxia. Increased BD > 20-25 mmol/L is associated with poor prognosis(4). In a study by Chile F et al (25) of a limited number of cases, it was emphasized that high CRP levels in PA patients were not related to infection, but no clear relationship was determined between high CRP levels and the severity of asphyxia. Okumus N et al (19) considered the high CRP levels to be related to the therapeutic hypothermia treatment. In the current study, the CRP level increased despite the negative blood culture results and absence of signs of clinical septicemia, and the increase in CRP level was related to BD, which is a sign of hypoxic birth, and it was determined that in cases with high BD at diagnosis, CRP levels were higher. In contrast to similar studies in literature, this suggested that the increase in CRP levels was related to the severity of PA rather than therapeutic hypothermia.

A limitation of this study was that magnetic resonance imaging (MRI) was not applied to all patients and so the elevation in CRP could not be related to brain damage on MRI. There is a need for further, multi-centre, randomised, controlled studies conducted on selected patient groups to confirm the effects of PA on infection markers.

CONCLUSION

The results of this study demonstrated that the CRP level in cases that developed asphyxia was significantly higher than that of the control group. A positive correlation was determined between BD at the time of diagnosis and CRP levels in PA cases. It can be considered that despite the effects of therapeutic hypothermia suppressing the immune response, high CRP levels can be used to support the diagnosis of PA and as findings supporting the severity of hypoxia.

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

Financial Disclosure: There is not any sources of financial assistance.

Ethical approval: This study conformed to the principles of the 2008 Declaration of Helsinki and was approved by the local ethics committee of Harran University, Medical Faculty, Turkey (Approval date/number: 07.01.2019/190102).

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REFERENCES

1. M Polat, A Şimşek, N Tansuğ, et al. Prediction of neurodevelopmental outcome in term neonates with hypoxic-ischemic encephalopathy. Eur J Paediatric Neurol 2013;17.3:288-93.
2. Nuñez A, Benavente I, Blanco D, et al. Oxidative stress in perinatal asphyxia and hypoxic-ischaemic encephalopathy. Anales de Pediatría 2018;88:228-9.
3. Papile LA, Baley JE, Benitz W, et al. Hypothermia and neonatal encephalopathy. Committee on Fetus and Newborn, Pediatrics 2014;133:1146
4. Kliegman Robert M. Hypoxic ischemic encephalopathy. Nelson Textbook Of Pediatrics (20th ed) Vol 1 IN: Nervous System Disorders, 2016;99.5:834-42.

5. Aluvaala J, Collins GS, Maina M, et al. A systematic review of neonatal treatment intensity scores and their potential application in low-resource setting hospitals for predicting mortality, morbidity and estimating resource use. Syst Rev 2017;6:248.
6. Hacımustafaoğlu M. Acute Phase Reactants (Erythrocyte Sedimentation Rate, CRP) J Pediatr Inf 2017;11:53-5
7. Perlman JM, Wyllie J, Kattwinkel J, et al. Part 11: neonatal resuscitation: 2010 international consensus on cardiopulmonary resuscitation and emergency cardiovascular care science with treatment recommendations. Circulation 2010;122:516-38.
8. Procianoy RS. Hipotermia terapêutica. SBP. Departamento de Neonatologia. Documento científico cited 2015.
9. Y Gao, R Fu, J Wang, et al. Resveratrol mitigates the oxidative stress mediated by hypoxic-ischemic brain injury in neonatal rats via Nrf2/HO-1 pathway. Pharm Biol 2018;56:440-9.
10. L Merrill. Therapeutic hypothermia to treat hypoxic ischemic encephalopathy in newborns. Nurs Womens Health 2012;16:126-34.
11. Papile LA, Baley JE, Benitz W, et al. Hypothermia and neonatal encephalopathy. Pediatrics 2014;133:1146
12. S McWilliam, A. Riordan. How to use: C-reactive protein. Arch Dis Child Educ Pract Ed 2010;95.2: 55-8.
13. Lally P J, Montaldo P, Oliveira V, et al. Residual brain injury after early discontinuation of cooling therapy in mild neonatal encephalopathy. Arch Dis Child Educ Pract Ed 2018;103:383-7.
14. Aibiki M, Maekawa S, Ogura S, et al. Effect of moderate hypothermia on systemic and internal jugular plasma IL-6 levels after traumatic brain injury in humans. J Neurotrauma 1999;16:225-32.
15. Chakkarapani E, Davis J, Thoresen M. Therapeutic hypothermia delays the C-reactive protein response and suppresses white blood cell and platelet count in infants with neonatal encephalopathy. Arch Dis Child Educ Pract Ed 2014;99:458-63.
16. Millar LJ, Shi L, Hoerder-Suabedissen A, et al. Neonatal hypoxia ischaemia: mechanisms, models, and therapeutic challenges. Front Cell Neurosci 2017;11:78.
17. Bonestroo HJC, Heijnen CJ, Groenendaal F, et al. Development of cerebral gray and white matter injury and cerebral inflammation over time after inflammatory perinatal asphyxia. Developmental Neuroscience 2015;37:78-94.
18. Wassink G , Gunn ER, Drury PP, et al. The mechanisms and treatment of asphyxial encephalopathy. Frontiers in Neuroscience 2014;8:40
19. Okumuş N, Beken S, Aydın B, et al. Effect of therapeutic hypothermia on C-reactive protein levels in patients with perinatal asphyxia. Am J Perinatol 2015;32:667-74.
20. Azzopardi DV, Strohm B, Edwards AD, et al. Moderate hypothermia to treat perinatal asphyxial encephalopathy. N Engl J Med 2009;361:1349–58.
21. Laptook AR, Shankaran S, Tyson JE, et al. Eunice kennedy shriver national institute of child health and human development neonatal research network effect of therapeutic hypothermia initiated after 6 hours of age on death or disability among newborns with hypoxic-ischemic encephalopathy: a randomized clinical trial. JAMA 2017;318:1550-60.
22. Archambault J, Moreira A, McDaniel D, et al. Therapeutic potential of mesenchymal stromal cells for hypoxic ischemic encephalopathy: A systematic review and meta-analysis of preclinical studies. PloS One 2017;12:e0189895.
23. Ahearne CE, Boylan GB, Murray DM. Short and long term prognosis in perinatal asphyxia: An update. World J Clin Pediatr 2016;5:67.
24. Papile LA, Baley JE, Benitz W, et al. Hypothermia and neonatal encephalopathy. Committee on Fetus and Newborn, Pediatrics 2014;133:1146
25. Chiale F, Di Sabatino F, Calzedda R, et al. Persistent High CRP levels in nonseptic asphyxiated newborns: A Report of Three Cases Amer J Perinatol 2018;35:1-26.