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Diagnostic and Prognostic Value of Serum NT-ProBNP in the Diagnosis of Neonatal Sepsis

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ABSTRACT

Despite the advances in the diagnosis and treatment of neonatal sepsis, it remains an important cause of morbidity and mortality. This study aimed to investigate the effectiveness of N-terminal pro-B-type natriuretic peptide (NT-ProBNP) levels in the diagnosis and prognosis of neonatal sepsis.

Fifty neonates diagnosed with clinical sepsis in the neonatal intensive care unit were included in the study. The control group was composed of 50 healthy neonates.

As a result of the study, a statistically significant difference was observed between the groups in terms of NT-proBNP, C-reactive protein, leukocyte count, platelet count and I/M ratio($p < 0.05$). NT-ProBNP level was 19624.1±15027.6 pg/ml in the case group, while it was 3203.8±4506.8 pg/ml in the control group. There was a positive correlation between NT-ProBNP and neonatal sepsis in the case group. NT-ProBNP measurements were found to be significant in differentiating neonatal sepsis. In the case group, 33 patients discharged with recovery, 17 patients died, and the mean NT-ProBNP levels were 12732.2±12954.3 pg/ml and 35000 pg/ml, respectively. NT-ProBNP levels were statistically significantly higher in died patients.

NT-ProBNP levels should be measured in the early diagnosis of neonatal sepsis and to determine the prognosis of patients diagnosed with neonatal sepsis. The use of NT-ProBNP with other biomarkers helps the early diagnosis of neonatal sepsis. Further multicenter, prospective studies with large samples are needed to identify NT-ProBNP levels in the diagnosis and prognosis of neonatal sepsis.

Keywords: Neonate, Sepsis, NT-proBNP

Introduction

Neonatal sepsis (NS) is a diagnosis that occurs in the first 28 days of life, including clinical conditions such as systemic infection symptoms, circulatory shock and multiorgan failure (1). Neonatal sepsis is divided into two groups as early-onset sepsis (ENS), diagnosed in the first 72 hours of life and with pathogens mostly acquired perinatally, and late-onset sepsis (LNS), diagnosed from the fourth day of life and with pathogens acquired from community or healthcare setting (2). The overall incidence of NS is between 1-5 per 1000 live births (3). Its incidence is higher in premature babies and those with very low birth weight (4). The immature immune system of neonates is an important factor contributing to the development of NS, and neonates cannot produce a complete inflammatory response (5). The clinical manifestation of NS is not specific, and clinical signs may be observed such as inadequate nutrition, food intolerance, dyspnea, pneumonia, apnea, prolonged capillary refill time, cutis marmoratus, necrotizing enterocolitis, hypothermia and hyperthermia, hypotonia, seizures, fontanel pressure, diffuse intravascular coagulation, bleeding and prolonged jaundice (6). Since NS signs and symptoms are not mild and specific, laboratory tests are performed in neonates with identifiable risk factors and/or sepsis-related signs and symptoms (7). Although blood culture is the gold standard in the diagnosis of NS, it takes a certain time(usually about 24 to 72 hours) to detect blood culture positivity, and its sensitivity is poor (1,8). Organisms that are difficult to produce, maternal antibiotics and small amounts of blood collection limit the sensitivity of blood cultures (1). Increased levels of various biomarkers including NT-ProBNP, C Reactive Protein (CRP), interleukin 4, interleukin 6, tumor necrosis factor alpha, procalcitonin and interleukin-10 also have high sensitivity for identifying infants with NS (1,2,6,9). Apart from blood culture, strategies are needed to improve the predictive ability to detect NS, as a specific finding or test does not reliably identify non-infected infants (1). B Type natriuretic peptide(BNP) is a neurohormone with diuretic, natriuretic and vasodilator effects, and it converts to BNP as the active peptide and to the biologically inactive NT-ProBNP (10). NT-ProBNP can be a useful biomarker

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to predict survival in patients with severe neonatal sepsis and can be used as an early predictor to show myocardial dysfunction in patients with neonatal septic shock (11,12). In this study, we aimed to investigate the effectiveness of N Terminal Pro-B Type natriuretic peptide levels in the diagnosis and prognosis of neonatal sepsis.

Materials and Methods

This study was conducted with the patients hospitalized in the neonatal intensive care unit with the pre-diagnosis of neonatal sepsis and healthy neonates who were admitted to our outpatient clinic or born in the obstetrics and gynecology service during the same period.

Study Groups: The study population consisted of 50 term and preterm neonates suspected of NS and 50 healthy neonates. Infants with a score of 5 or above according to the Töllner sepsis scoring system (13) and diagnosed with suspected NS, possible sepsis and confirmed sepsis in the first 28 days were included in the study. Patients with chromosomal disorders, major congenital anomalies and undergoing surgery were excluded from the study. A "patient follow-up form" was filled in for each patient. Before starting empirical antibiotherapy in neonates suspected of NS and with a Töllner score of 5 or above, 1 cc of blood was taken into an EDTA tube for NT-ProBNP analysis as well as routine examinations and studied at the biochemistry laboratory at all hours. In addition, blood culture was taken from each patient, and urine, CSF and other cultures (umbilical swab, rectal swab, tracheal aspirate, etc.) were taken, if required, based on the clinical picture of patient. Lumbar puncture was performed in the patients with signs of meningitis. Chest radiographs were obtained in the patients suspected of NS and with respiratory symptoms. Hemogram, peripheral smear and blood gases required for the Töllner sepsis scoring were evaluated in each patient. Clinical sepsis diagnosis was made in the patients with at least 2 of the criteria listed below, provided that they had NS signs and symptoms.

A Töllner score of ≥ 5

Presence of hematological findings(leukocytosis, leukopenia, thrombocytopenia, an I/M(immature mature neutrophil) ratio of ≥0.2)

Increased levels of CRP

Neonates included in the case group

Patients hospitalized in the neonatal unit with the pre-diagnosis of neonatal sepsis without any cardiac or any others serious problems.

Those not having received antibiotic therapy

Patients diagnosed with NS while hospitalized

NT-ProBNP level was examined only at the time of hospitalization in the case group, while the control group was taken from babies who were completely healthy in age and weight similar to the case group.

Neonates included in the control group

Those born from healthy mothers.

Those without a history of EMR, chorioamnionitis or meconium aspiration

Those not having received antibiotic therapy

Those without a history of perinatal asphyxia

Neonates without postpartum problems

Symptoms such as poor feeding, fever, toxic appearance, lethargy, irritability, cyanosis, tachypnea, dyspnea, convulsions, vomiting, abdominal distention and hypotonia were evaluated in favor of infection. Clinical findings were evaluated by the physician who conducted the study during hospitalization. The case group was divided into two subgroups as recovered patients and died patients.

Laboratory Examinations

Blood culture: Blood culture was taken from each case before starting antibiotherapy. 0.5-1ml of venous blood was inoculated into the pediatric BACTEC culture media. The media were placed in the oven of BACTEC 9120 (Becton Dickinson, USA) hemoculture device. The samples were daily checked for reproduction.

Hemogram: Approximately 1 cc of venous blood was taken into a K3 EDTA tube for the detection of hematological parameters such as leukocyte count, hemoglobin and platelet. Hemogram analysis was performed automatically on the Beckman Coulter LH 780 device.

Peripheral Smear: A drop of blood taken from the fingertips of neonatal patients was placed on the slide, smeared and dried. After stained with May-Grunwald and Giemsa, peripheral smears were examined by microscope at 100x magnification. An I/M ratio of ≥0.2 was considered pathological.

CRP levels were determined quantitatively using an immune nephelometric method and appropriate kit on the Beckman Coulter device. The values above 5 mg/dL were considered significant.

NT-ProBNP level: Approximately 1 cc of venous blood was taken into a K3 EDTA tube and

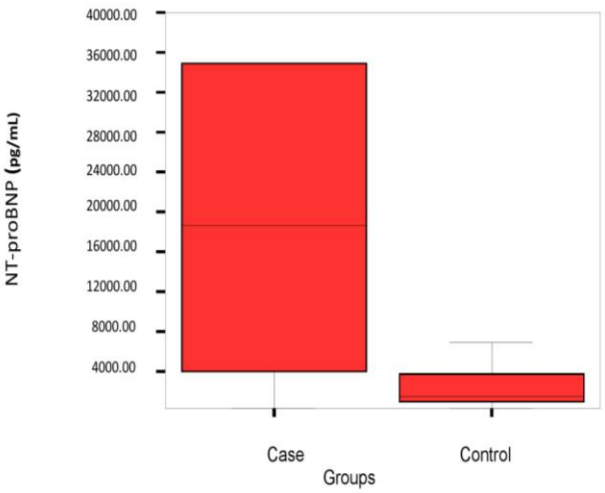

Fig. 1. Distribution of NT-ProBNP levels between the case and control groups

transported to the laboratory on ice. The blood was centrifuged at 3500 rpm for 5 minutes, and the serum was separated. The serum sample was measured with a chemiluminescent immunometric assay method, using the CIRRUS diagnostic inc.(Los Angeles, USA) device and Immulite 1000 Turbo, NT pro-BNP (PILSKNT-17,2008-05-30) kit at the biochemistry laboratory.

Statistical Analysis: This study was conducted as a prospective study. The data of infants included in the study were recorded to previously prepared standard forms. These data were uploaded to the computer and processed with Statistical Package for Social Sciences for Windows 18 software. Means and histograms were calculated. Kolmogrov-smirnov normality test was used. The Student's t-test was used in the comparison of control group with the case group. A p value of <0.05 was considered statistically significant. Informed consent was obtained from the families of infants.

Results

A total of 100 neonates, in the case group 27 girls(54%) and in the control group 22 girls(44%), were included in the study. The mean gestational week in the case group was 36.7 weeks and in the control group 38.2 weeks. The mean chronological age was 11.3 days in the case group and 9.1 days in the control group. No demographic differences were observed between the groups(p0.065, p0.203, p0.424, respectively).

Of laboratory examinations, the mean leukocyte count, platelet count, I/M(immature/mature leukocyte ratio) ratio and CRP were found to be statistically significantly higher in the case group compared to the control group, with the values of 16817 /mm^3, 186.000/mm^3, 23.9% and 57.9 g/L, respectively(p=0.02, p=0.01, p=0.01, p=0.001, respectively)(Table 1). The mean Hb value was 15.5 g/L, and there was no statistically significant difference between the groups(p=0.45) (Table 1).

The mean NT-ProBNP levels were 19624.1±15027.6 pg/ml in the case group and 3203.8±4506.8 pg/ml in the control group and were significantly higher in the case group compared to the control group($p < 0.001$).

When the mean NT-ProBNP levels of 33(66%) recovered and 17(34%) died patients in the case group were compared, the mean NT-ProBNP level was 12732.2±12954.3 pg/ml in recovered cases, while it was 35000 pg/ml in died cases, and a statistically significant difference was observed between the groups ($p < 0.001$).

The control group was divided into 4 subgroups by age, and NT-ProBNP levels were determined accordingly to find references value. When Table 2 and Figure 1 were examined, the highest NT-ProBNP levels in the neonatal period were observed in the first 3 days of life and gradually decreased with age. Based on the relevant literature, no normal NT-ProBNP levels are observed between 12-30 days of life, and the mean NT-ProBNP level of this age group was found to be 1639.1±2462.4 pg/ml in our study.

Discussion

Neonatal sepsis is an emergency situation that causes high morbidity and mortality (14). Although a long period of time has passed since the clinical definition of neonatal sepsis, various difficulties are still encountered in early diagnosis. Despite the large number of screening tests used in the early diagnosis of NS, the sensitivity of these tests ranges from 30% to 90%(15). Blood culture is the gold standard for diagnosing NS, however, it is a condition that causes a certain time to pass with a low sensitivity, insufficient amount of blood collection and generally false negative results (16,17). Due to various restrictions on hematological and microbiological techniques routinely used for the early diagnosis of NS, ideal biomarkers are needed to ensure early, specific and reliable identification of newborns at high risk of infection. The advancement of technological advances and the use of biomarkers are increasing. A better understanding of the neonatal immune system have enabled the identification of potential

Table 1. Evaluation of Laboratory Findings

	Case Group (n =50)	Control Group (n=50)	p
	Mean±SD	Mean±SD	
Leukocyte count(mm³)	16817±13204	12321±5164	0.02+
Hemoglobin(g/dL)	15.5±3.2	15.9±2.5	0.45+
Platelet count(mm³)	186.000±142.56	273.100±100.405	0.01+
I/M ratio (%)	23.9±10.9	9.2±6.7	0.01+
CRP (mg/L)	57.9±79.8	3.1±1.8	0.001+

+Student's t-test, *I/M: Immature/Mature leukocyte, CRP: C-reactive-protein*; SD: Standart Deviation

Table 2. Mean NT-ProBNP values of the control group

Age	n	Mean ± SD
0-1 days	4	8300.2±7398.9
2-3 days	9	6367.4±7508.7
4-11 days	23	2032.0±1370.8
12-30 days	14	1639.1±2462.4
Total	50	3203.8 ± 4506.9

SD: Standart Deviation

biomarkers that could lead to early diagnosis of neonatal sepsis in the coming years (18).

Brain Natriuretic Peptide, plays an important role in the regulation of blood pressure during fetal development and natriuretic and diuretic changes during transition to postpartum life. There is a dramatic increase in NT-ProBNP levels in the early postnatal period (19). It is recognized that a significant increase of natriuretic peptides is required for diuresis and natriuresis for the first few days after birth, and it should be borne in mind that the most important changes of NT-ProBNP occur in the first 48 hours of life and decrease to normal values within 1 week after birth (19). A meta-analysis including 35 studies on adult patients showed that the patients with sepsis had elevated BNP/NT-ProBNP levels, the optimal cut-off values were not fully identified for mortality prediction, but 622 pg/mL-4000 pg/mL BNP and NT-ProBNP levels should be used for mortality prediction (10). Few studies have investigated the relationship between neonatal sepsis and NT-ProBNP in the literature. A pediatric study found a significant difference between survivors and non-survivors of sepsis in terms of baseline NT-ProBNP levels(6280.3±9597 ng/L, $p < 0.001$) (25). A baseline NT-ProBNP level more than 11200 pg/ml had the sensitivity of 85.7% and specificity of 90% to predict pediatric intensive care unit (PICU) mortality. High baseline NT-ProBNP level was found to be associated with mortality. This may indicate the severity of infection associated with high NT-ProBNP level. Baseline plasma NT-ProBNP levels were found to be significantly higher in all septic patients and non-survivors compared to survivors. Baseline plasma NT-ProBNP levels have been shown to be an independent prognostic predictor of mortality (20). Another study reported that BNP level at admission to the intensive care unit was associated with mortality and might play a role in directing fluid therapy in septic patients (21). A neonatal study found that plasma NT-ProBNP levels were higher in septic neonates, and this parameter had the sensitivity of 72%, specificity of 86% and high predictive value (22). An adult study demonstrated the use of NT-ProBNP and CRP as independent predictors of mortality in septic patients over 75 years of age in the intensive care unit and that the incorporation of NT-ProBNP or CRP or both into the APACHE II score significantly improved the ability to predict intensive care unit mortality (23). When NT-ProBNP levels were compared between the groups, the mean NT proBNP levels were 19624.1±15027.6 pg/ml in the case group and 3203.8±4506.8 pg/ml in the control group and were statistically significantly higher in the case group compared to the control group ($p < 0.001$). Considering our results and other current studies, we think that NT-ProBNP will be a useful marker in the early diagnosis of neonatal sepsis.

Han et al. demonstrated that early and aggressive hemodynamic resuscitation is important in children with septic shock and reported that there is a relationship between early treatment of pediatric-neonatal septic shock and better outcomes(24). The mean NT-ProBNP levels of 33(66%) recovered patients and 17(34%) died patients in the case group were found to be 12732.2±12954.3 pg/ml and 35000 pg/ml, respectively, and there was a statistically significant difference between the two groups ($p < 0.001$). Based on these results, it can be said that higher NT-ProBNP level indicates poorer prognosis. It was thought that NT-ProBNP could be a useful marker in determining the prognosis of neonatal sepsis, and more aggressive treatment should be provided in patients with high NT-ProBNP levels.

C-Reactive Protein, is the most commonly used laboratory test in neonatal sepsis (15). Most of the studies revealed that CRP levels were of importance in the diagnosis and follow-up of neonatal sepsis (14,25,26). In our study, the mean serum CRP values were 57.9 mg/L in the case group and 3.1 mg/L in the control group, and were statistically significantly higher in the case group compared to the control group($p < 0.000$) (Table 1).

There are many studies in the literature investigating the relationship of neonatal sepsis with thrombocytopenia and hematological parameters. Thrombocytopenia, I/M ratio, I/T ratio and total leukocyte count are examined as parameters in the Rodwell and Töllner scoring systems used in the early diagnosis of sepsis (27). Guida et al. found that 54% of neonates with very low birth weight and diagnosed with neonatal sepsis had thrombocytopenia in their study (28). In other studies, platelet count was found to be lower in patients with neonatal sepsis compared to the control group (29,30). Lal et al. determined the sensitivity of hematological scoring system (HSS) as 86.95%, specificity as 78.12%, and positive predictive value (PPV) as 74.07% in predicting sepsis by using I/M ratio, I/T ratio and total leukocyte count in the hematological scoring system (30). In our study, the mean platelet count was 186.000 /mm³ in the case group, 273.100 /mm³ in the control group, the mean I/M ratio was 23.9% in the case group, and these parameters were found to be significant in the diagnosis of sepsis (Table 1) ($p < 0.01$). When used with other parameters, it was found that thrombocytopenia and I/M ratio may also be significant in the diagnosis of sepsis.

As a result of the study, NT-ProBNP levels was found to be statistically significantly higher in neonates with neonatal sepsis compared to the control group. The mean NT ProBNP levels were measured as 19624.1±15027.6 pg/ml in the case group and 3203.8±4506.8 pg/ml in the control group. The mean NT-ProBNP levels were measured as 19624.1±15027.6 pg/ml in recovered cases, and 35000 pg/ml in died cases, and NT-ProBNP was found to be statistically significantly higher in died cases compared to recovered cases. In the light of these results, NT-ProBNP is a practical test that can be used with other diagnostic markers of infection in the diagnosis and prognosis of neonatal sepsis.

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