N-terminal pro-brain natriuretic peptide as a biomarker for differentiating cardiac and pulmonary disease in term neonates with respiratory distress

N-terminal pro-brain natriuretic peptide as a biomarker for differentiating cardiac and pulmonary disease in term neonates with respiratory distress

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Abstract

Objective: Brain natriuretic peptide (BNP) is synthesized in the cardiac ventricles and released in response to volume or pressure load. The aim of the study was to determine whether plasma level of N-terminal pro BNP (NT-pro BNP) can distinguish between cardiac and pulmonary disease (PD) among neonates with respiratory distress (RD).

Patients and methods: The study included 48 term neonates in the first month of life with signs of RD. They were recruited from Neonatal Intensive Care Unit of Al-Galaa Teaching Hospital. Twenty-six healthy neonates were included as a control group. The degree of RD was assessed using Silverman−Anderson score. Chest X-ray, echocardiography, and laboratory measurement of NT-pro BNP were performed.

Results: According to the underlying disease, neonates with RD were divided into 28 neonates with PD and 20 neonates with congenital heart disease (CHD). Regardless the etiology of RD, NT-pro BNP was significantly higher in the RD group than in the control ($p = 0.001$). There was a significant difference between and within the three groups regarding NT-pro BNP ($p = 0.001$). NT-pro BNP was significantly higher in the CHD group than in the PD group ($p = 0.001$). There was a significant difference between and within RD subgroups. The NT-pro BNP is a very useful test for identification of CHD in neonates with RD. Area under the receiver operating characteristic curve for CHD was 0.857 ($p = 0.01$), sensitivity 66%, specificity 85%, and cutoff point was 24.5 pg/mL. The area under the curve for PD was 0.646 ($p = 0.1$) with poor sensitivity and specificity, indicating that NT-pro BNP is a poor test for identification of PD in neonates with RD.

Conclusion: Term neonates with RD have increased plasma levels of NT-pro BNP. NT-pro BNP is a very good test for identification of CHD in neonates with RD, in comparison with PD. Therefore, plasma NT-pro BNP can be used to differentiate between cardiac and pulmonary cause of RD.

Keywords: Congenital heart disease, Neonates, NT-pro BNP, Respiratory distress

1. Introduction

Natriuretic peptides are members of cardiac biomarkers that provide an overview for the structure and functioning of the heart in the newborn infants [1].

Brain natriuretic peptide (BNP) is synthesized in the cardiac ventricles and released in response to volume or pressure load [2]. Therefore, it can be used as a tool for differentiation between cardiac and non-cardiac cause of acute dyspnea [3].

Pro-BNP which is the inactive precursor is splinted into BNP "active component" and N-terminal pro BNP (NT-pro BNP) "inactive end product" [4]. Several researches have shown a significant correlation between BNP and NT-pro BNP [2].

Deficiency of surfactant is the most common cause of respiratory distress (RD) in preterm neonates.

Meanwhile, in term neonates, RD can be caused by several other problems, many of which are cardiovascular [5].

In developing countries, echocardiography is unavailable in all centers and routine investigations (e.g., chest radiography) are insufficient for diagnosis of congenital heart disease (CHD). Therefore, cardiac biomarkers could be of great help [6].

This study aimed to determine whether plasma level of NT-pro BNP can distinguish between cardiac and pulmonary disease (PD) in neonates with RD.

2. Patients and methods

2.1. Participants

This study included 48 term neonates (28 males and 20 females) in the first month of life. Neonates showed signs of RD. They were recruited from Neonatal Intensive Care Unit of Al-Galaa Teaching Hospital. Exclusion criteria included neonates with congenital anomalies, such as cleft palate and abnormal faces and limbs denoting genetic syndromes. Twenty-six healthy neonates were included in the study as a control group (12 males and 14 females). Parental consent for all neonates was obtained according to the form approved by the Ethics Committee of the National Research Center.

2.2. Methods of assessment

We collected demographic data such as gestational age, mode of delivery, sex, birth weight, and Apgar score for each neonate enrolled in the study. The degree of RD was assessed using Silverman—Anderson score [7]. A score of 1—3 was considered to indicate mild, 4—6 moderate, and 7—10 severe RD. Chest radiography and echocardiography were performed for every neonate in the RD groups.

2.3. Echocardiographic examination

Echocardiographic studies were performed with a Vivid 3 Expert (Norway) using 7 MHz transducers. Two-dimensional views were used to detect cardiac septal defects. For accurate measurement of these defects, the direction of the shunt color flow mapping was obtained. Doppler examinations were performed to assess the pulmonary blood flow. The images were obtained according to the usual standardization [8].

2.4. Laboratory investigations

From each participant, 3 mL of venous blood was collected and the samples were divided into two parts: (1) 1.5 mL of venous blood was added into a tube containing EDTA for complete blood count (CBC) determination and (2) 1.5 mL of the remainder blood was allowed to clot for 30 minutes before centrifugation for 15 minutes at $1000g$; the serum was removed and assayed immediately or aliquot and stored at -20 °C.

The CBC was performed on coulter counter T890 (Coulter counter, Harpenden, UK). Serum NT-pro BNP was determined using sandwich enzyme-linked immunosorbent assay kit (Abnova, Walnut, CA, USA).

2.5. Statistical analysis

SPSS version 16 (SPSS Inc., Chicago, IL, USA) was used for the analysis of data. Data were summarized as mean ± standard deviation. Student t test for quantitative independent variables was used for the analysis of difference between two groups. Comparison of multiple subgroups was done using one-way analysis of variance (ANOVA).

Correlation between quantitative variables was done using Pearson's bivariate test. A p value < 0.05 was considered statistically significant.

Receiver operator characteristic (ROC) curves were drawn for CHD and PD groups, and the area under the curves (AUCs) were calculated for the correlation between levels of NT-pro BNP and RD.

3. Results

In total, 48 term neonates with RD were included in the study. They were divided into 28 neonates with PD and 20 neonates with CHD according to underlying diseases.

Characteristics of neonatal groups are summarized in Table 1. No significant differences were found between the three groups regarding gestational age, birth weight, postnatal age, hematocrit percentage, and platelet count ($p > 0.05$). Meanwhile, there was significant leukopenia in PD group compared with the control group ($p = 0.02$).

Abbreviations

ANP	Atrial natriuretic peptide
BNP	Brain natriuretic peptide
CBC	Complete blood picture
CHD	Congenital heart disease
NICU	Neonatal intensive care unit
NT-proBNP	N Terminal pro brain natriuretic peptide
PDA	Patent ductus arteriosus
RD	Respiratory distress

Table 1. Characteristics of neonatal groups.

Characteristics	Pulmonary disease ($n = 28$)	Congenital heart disease ($n = 20$)	Control ($n = 26$)	p
Gestational age (wk)	35.79 ± 2.5	37.2 ± 1.36	36.69 ± 1.08	p_1 0.09 p_2 0.1
Birth weight (kg)	2.71 ± 0.6	2.95 ± 0.41	2.93 ± 0.28	p_1 0.08 p_2 0.9
Postnatal age (d)	2.43 ± 1.13	3.8 ± 1.76	3.31 ± 2.13	p_1 0.06 p_2 0.4
Hematocrit (%)	34 ± 2.62	33.6 ± 3.3	34.3 ± 1.89	p_1 0.6 p_2 0.3
Platelets/μL	1.887 ± 70.46	2.006 ± 87.15	2.11 ± 42.3	p_1 0.1 p_2 0.5
Leukocytes/μL	1.406 ± 9.37	1.104 ± 3.69	9.5 ± 2.91	p_1 0.02[*] p_2 0.1

$p1$ = comparison of pulmonary disease group versus control; $p2$ = comparison of congenital heart disease group versus control.
[*] A p value < 0.05 is significant.

Causes of RD in the studied neonates are demonstrated in Table 2. Regardless of the etiology of RD, NT-pro BNP was significantly higher in the RD group than in the control group (19.98 ± 7.19 vs. 8.07 ± 1.02; $p = 0.001$; Fig. 1).

Comparison between the studied neonatal groups revealed that there was a highly significant difference between and within the three groups regarding the mean of NT-pro BNP (NT-pro BNP mean of CHD, PD, and control was 27.03 ± 5.28, 14.95 ± 2.72, and 8.07 ± 1.02, respectively; $p = 0.001$), i.e., NT-pro BNP was significantly higher in the CHD group than in the PD and control groups (Fig. 2).

Furthermore, the RD group was divided according to the degree of RD into mild, moderate, and severe RD subgroups (Table 3).

Analysis using the ANOVA test shows that there was a highly significant difference between and within RD subgroups regarding the mean of NT-pro BNP ($p = 0.001$; Table 3 and Fig. 3).

The ROC curve is plotted with NT-pro BNP as a test variable and degree of RD as a state variable. The AUC for CHD is 0.857 ($p = 0.01$), the cutoff point is 24.5 pg./mL, sensitivity 66%, specificity 85%, positive predictive value 87%, and negative predictive value 50%, indicating that NT-pro BNP is a very useful test for identification of CHD in neonates with RD.

The AUC for PD was 0.646 ($p = 0.1$) with poor sensitivity and specificity, thus indicating that NT-pro BNP is a poor test for identification of PD in neonates with RD.
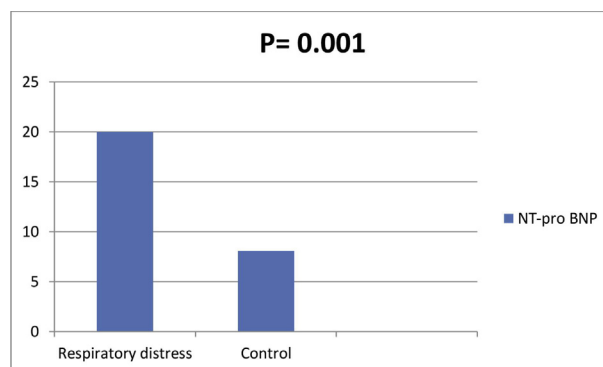

Fig. 1. Measurement of NT-pro BNP in the respiratory distress group and control. NT-pro BNP = N-terminal pro-brain natriuretic peptide.
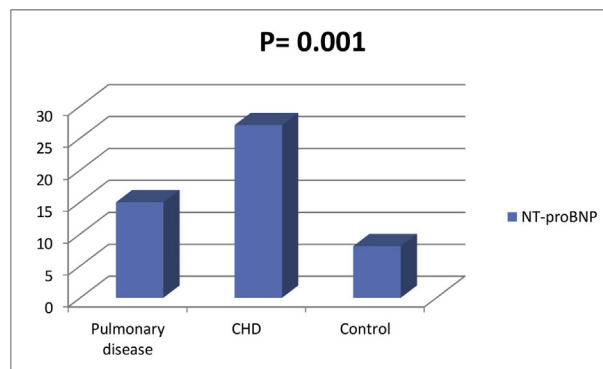
Table 2. Causes of respiratory distress in the studied neonates.

| Diagnosis	Patients (n)
Pulmonary diseases group	28
Transient tachypnea	14
Meconium aspiration syndrome without PH	8
Aspiration pneumonia	6
Congenital heart disease group	20
Large PDA with moderate PH	10
Small PDA with VSD	4
Small PDA with small ASD and moderate PH	6

ASD = atrial septal defect; PDA = patent ductus arteriosus; PH = pulmonary hypertension; VSD = ventricular septal defect.

Fig. 2. Measurement of NT-pro BNP in the three studied groups. NT-pro BNP = N-terminal pro-brain natriuretic peptide.

Table 3. Respiratory distress group according to severity.

Category	Patients (n = 48)	Birth weight, kg (mean ± SD)	Gestational age, wk (mean ± SD)	N-terminal pro-BNP
Mild RD	8	2.5 ± 1.02	35 ± 2	11.35 ± 0.64
Moderate RD	18	2.9 ± 0.36	36.5 ± 2.7	17.93 ± 4.05
Severe RD	22	2.75 ± 0.39	36.7 ± 1.5	24.8 ± 6.79

BNP = brain natriuretic peptide; RD = respiratory distress.

A highly significant positive correlation was revealed between NT-pro BNP in compassion to respiratory rate of the studied RD groups ($p = 0.001$, $r = 0.69$; Fig. 4).
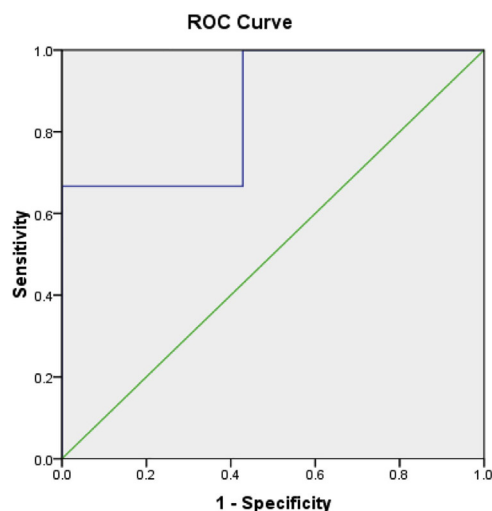
(A) Congenital heart disease (CHD).
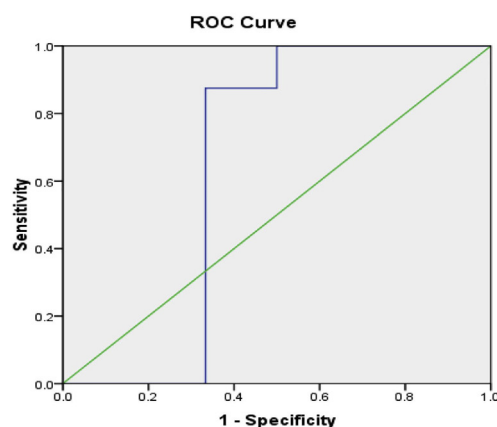
(B) Pulmonary disease.

Fig. 3. Receiver operating characteristic (ROC) curves for (A) neonates with congenital heart disease and (B) neonates with pulmonary disease. N-terminal pro-brain natriuretic peptide as test variable and degree of respiratory distress as state variable. Area under curves = 0.857 for congenital heart disease and 0.646 for pulmonary disease.

4. Discussion

In neonates, RD remains the most common cause of morbidity, mortality, and extra health care costs [9]. This study included 48 term neonates with RD aged 1–30 days. They were divided into neonates with PD ($n = 28$) and neonates with CHD ($n = 20$).

Regardless the etiology of RD, NT-pro BNP was significantly higher in the RD group than in the control group (19.98 ± 7.1 vs. 8.07 ± 1.02; $p = 0.001$).

Similar to our study, Markovic-Sovtic et al. [2] found that in their study, comprising 62 term neonates aged 1–28 days with signs of RD, the mean of NT-pro BNP level was significantly higher than in those without any signs of RD (9.65 ± 1.13 vs. 8.2 ± 1.1; $p = 0.001$).

Shahramian et al. [10] conducted a case-control study involving 65 preterm infants aged <37 weeks with Apgar score >7 as cases and 65 term infants as controls and demonstrated that serum BNP level increased among RD cases.

Furthermore, this fact has been explored in preterm neonates in prediction of RD in various studies [11,12].

Natriuretic peptides have an important role in the regulation of extracellular fluid volume and blood pressure [13]. They antagonize the effects of stress hormones such as those produced by the renin-angiotensin aldosterone system and induce natriuresis, diuresis, and vasodilatation [14].

The cardiac natriuretic peptides are either A-natriuretic peptide (ANP) or B-natriuretic peptide (BNP). ANP is secreted from the cardiac atria in response to increased left and right atrial pressure and volume loads, whereas BNP is secreted from the ventricles in response to increased left and right ventricular pressure and volume loads [15].

In the present study, neonates with underlying CHD had significantly higher NT-pro BNP than those with underlying PD ($p = 0.001$). Therefore, our study suggests that plasma NT-pro BNP measurements could differentiate between cardiac and pulmonary cause of RD in term neonates.

Similarly Cohen et al. [16] found that in their study, comprising infants (median age, 10 months) who presented with RD, plasma NT-pro BNP levels
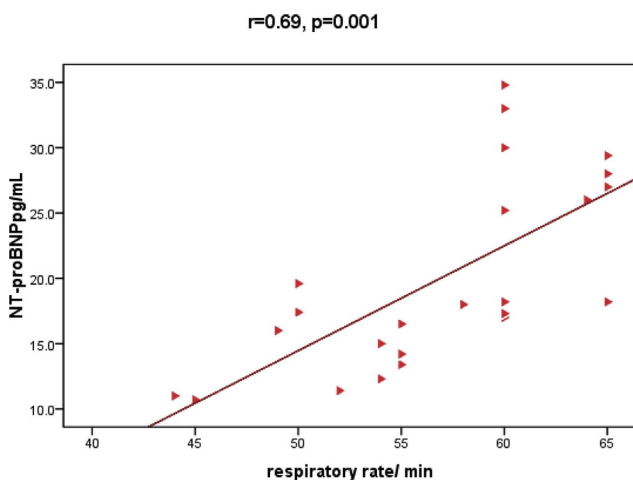
r=0.69, p=0.001

Fig. 4. Correlation between NT-pro BNP and respiratory rate of the studied respiratory distress groups. NT-pro BNP = N-terminal pro-brain natriuretic peptide.

were statistically higher in infants with heart disease than in those with respiratory disease. Also, Kou-louri et al. [5] reported in a study involving 49 in-fants and children presenting with acute RD that BNP level can distinguish cardiac from pulmonary causes of RD [17].

Several studies [17—19] studied the natriuretic peptide level in children with heart disease. They found that increased peptide levels were present in children with heart failure from structural heart disease as well as those with dilated cardiomyopa-thy and reported that patients with RD due to heart disease had significantly higher plasma NT-pro BNP levels than patients with RD due to pulmonary disease or controls.

Meanwhile, Markovic-Sovtic et al. [2] found in their study that the mean of NT-pro BNP level was 9.72 ± 1.2 and 9.54 ± 1.04 in the CHD and PD groups, respectively. These values were not signifi-cantly different ($p = 0.5$). This was in agreement with Markovic-Sovtic et al. [2] who found the same results and reported that NT-pro BNP cannot differentiate between CHD and PD, which is con-trary to our findings.

In the current study, the RD group was divided according to the severity of RD. There was a highly significant difference between and within RD sub-groups ($p = 0.001$). Markovic-Sovtic et al. [2] showed that mean NT-pro BNP was significantly lower in the mild RD group than in the moderate ($p = 0.04$) or severe RD group ($p = 0.07$). This is in agreement with our findings.

In our study, AUC for CHD was 0.857 ($p = 0.01$), sensitivity was 66%, specificity was 85%, and cutoff point of NT-pro BNP was 24.5 pg/mL. Therefore,

NT-pro BNP proves to be a very useful test for CHD identification.

Bao et al. [20] reported on 60 neonates suffering from asphyxia, a group with myocardial injury, and another without cardiac injury. In concordance to our results, their AUC was 0.80 ($p = 0.001$), sensi-tivity was 83.3%, specificity was 80.5%, and cutoff point was 36.5 pg/mL. They concluded that serum NT-pro BNP level can reflect myocardial injury in neonates with asphyxia.

Similarly, Nielsen et al. [21] found that in their study, including 300 patients complaining of dys-pnea, NT-pro BNP levels in patients with heart failure were significantly higher and AUCs for males and females were 0.93 and 0.90, respectively; they also reported that NT-pro BNP seems prom-ising for diagnosis of heart failure and may reduce the need for echocardiographic screening with 50%.

In our study, the CHD group had patent ductus arteriosus (PDA). Experimental studies have shown a possible association between BNP and postnatal PDA. Ductal tissue from fetal and newborn mice shows a changing pattern of expression of natri-uretic peptide receptors [22]. Fetal ductal tissue has a higher ratio of natriuretic peptide receptors-A/B which maintain the ductal patency to natriuretic peptide receptors-C (responsible for degradation); this ratio reverses in the PDA of the normal newborn mice suggesting that vasodilator effects in the fetus are reduced after birth. These findings suggest the possible pathological role of BNP in maintaining PDA after birth [23].

The BNP has established itself to be useful in diagnosis, prognosis, and management of cardiac disease [24].

The 2013 American College of Cardiology/Amer-ican Heart Association Guideline for the manage-ment of heart failure reported that both serum suppression of tumorigenicity-2 (ST2) and Galectin-3 are novel biomarkers, which may add additional prognostic value over natriuretic peptides [25].

Meanwhile, Parker et al. [26] found that ST2 and Galectin-3, unlike BNP, are not developmentally regulated and therefore not confounded by age. Furthermore, the biologic and analytic variability of ST2 is much lower than BNP, suggesting that ST2 levels may be better in risk stratification and prognostication.

Our study includes the following limitations: (1) the sample size is too small to study data separately for each type of CHD; (2) this study includes pa-tients with PDA, atrial septal defect, and ventricular septal defect, but does not include patients with other forms of moderate and severe CHD; and (3) our sample set comprises term neonates with lack of

other pediatric age groups. These limitations require further examination with a large sample size.

5. Conclusion

The term neonates with RD have increased levels of plasma NT-pro BNP. NT-pro BNP is a very good useful test for identification of CHD in neonates with RD, in comparison with PD. Therefore, plasma NT-pro BNP can be used to differentiate between cardiac and pulmonary causes of RD in neonates.

Acknowledgments

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