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LEVELS OF URINARY BIOMARKERS FOR EARLY PREDICTION OF BRONCHOPULMONARY DYSPLASIA

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Abstract
This study examined the potential predictive value of urine levels of N-terminal pro-brain natriuretic peptide (NT-pro BNP) and 8-hydroxy-2'-deoxyguanosine (8-OHdG) for bronchopulmonary dysplasia (BPD) in preterm newborns. Of the 84 preterm children participating in this prospective cohort trial, 70 went on to develop BPD. We assessed the levels of NT-proBNP and 8-OHdG in the urine between day 7 and day 28 of life. Next, we used correlations between the two biomarkers and receiver operating characteristic curves to evaluate the prediction efficiency. Between DOL 7 and 28, the BPD group's levels of 8-OHdG and NT-pro BNP were much greater than those of the control group ($P < 0.05$). Furthermore, from DOL7 to DOL28, there was a significant correlation between the 8-OHdG level and the NT-proBNP level ($P < 0.001$), as well as a positive correlation between the 8-OHdG and NT-proBNP levels and the length of mechanical ventilation and oxygen exposure time ($P < 0.05$). Furthermore, there was a strong correlation ($P < 0.05$) between the development of BPD and the levels of NT-proBNP (DOL 7-28) and 8-OHdG (DOL 14-28). Urine 8-OHdG concentrations from DOL 14 to 28 and NT-pro BNP concentrations from DOL 7 to 28 may serve as useful non-invasive indicators of the development of BPD in preterm children.

KEYWORDS: Bronchopulmonary dysplasia, N-terminal pro-brain natriuretic peptide, and 8-hydroxy-2'-deoxyguanosine.

Introduction

Disruptions to the development of the lungs during pregnancy and damage to the developing lungs after birth result in bronchopulmonary dysplasia (BPD)[1]. As a result, the incidence of BPD increases with decreasing gestational age (GA). The frequency of BPD in neonatal critical care units is still significant despite advances in perinatal treatment, including the use of postnatal surfactants, prenatal corticosteroids, and moderate mechanical breathing techniques. Thus, the search for non-invasive, trustworthy, and practical predictive biomarkers for the early clinical diagnosis and identification of BPD is vital[2]. Currently, machine learning-based predictive models and biomarker identification are used as early BPD prediction techniques. Interleukin-6, carboxyhemoglobin, lipid hydroperoxide, glutathione, and tracheal aspiration (TA) fluid and bronchoalveolar lavage fluid (BALF) are among the recognised indicators found in serum[3]. Additionally, the machine learning models that are now used to predict early BPD incorporate perinatal variables, clinical data, genomes, proteomics, and metabolomics[4]. On the other hand, repetitive blood collection can harm the skin's and mucous membranes' integrity, raising the risk of infection in premature babies, and getting BALF via a bronchoscope greatly intensifies a newborn's suffering[5]. Moreover, high detection technology is needed for the prediction models, which is incompatible with routine clinical practice at primary hospitals. In contrast to blood and BLAF samples, urine testing is affordable, non-invasive, convenient, and enables repeated readings[6&7]. Therefore, it may be a better choice.

Oxidative stress has been linked to the incidence of BPD, according to several research. The most severe form of oxidative stress is DNA damage, and the most prevalent marker of DNA damage is 8-hydroxy-2'-deoxyguanosine (8-OHdG)[8]. Because they produce reactive oxygen species (ROS) more quickly and have less antioxidant protection, preterm newborns respond to oxidative stressors with reduced antioxidant defences[9]. Lung epithelial damage is mediated by this oxidative-antioxidant imbalance, which results in BPD[10]. Consequently, preterm newborns are more susceptible to lung damage caused by oxidative stress, particularly when they are receiving continuous mechanical breathing or oxygen support[11].

The very stable, physiologically inert N-terminal portion of B-type natriuretic peptide (BNP), known as N-terminal pro-brain natriuretic peptide (NT-proBNP), is released by cardiomyocytes at a low clearance rate[12]. Moreover, the kidneys and liver help the body get rid of NT-proBNP. A common method for evaluating heart failure in adults, kids, and newborns is plasma NT-proBNP[13]. Elevated blood NT-proBNP concentrations have been linked to preterm delivery problems such as hemodynamically significant patent ductus arteriosus (PDA), diaphragmatic hernia, and respiratory distress syndrome (RDS) in recent preterm newborn investigations[14]. The emergence of BPD is intimately linked to these issues.

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Furthermore, blood and urine had similar NT-proBNP contents. In order to determine if preterm baby urine 8-OHdG and NT-proBNP levels may serve as non-invasive biomarkers for BPD, this prospective cohort research examined these values.

Materials and methods

Choosing patients with bronchopulmonary dysplasia from January 2017 to January 2019, we prospectively performed prospective research on preterm neonates admitted to Department of Paediatrics, Mayo Institute of Medical Sciences. Faizabad Road.Barabanki. Lucknow ; 226010. Uttar Pradesh. India and admitted to the neonatal unit within the first week following delivery and having a GA of less than 32 weeks and a birth weight of less than 1,500 g. In addition, every parent provided informed permission. Entire medical records, discharge or death before 36 weeks postmenstrual age, twins or multiple infants, severe congenital heart disease, chromosomal disorder, hereditary metabolic disease, and other serious deformities were among the exclusion criteria[14].

BPD and severity were determined using the 2018 Workshop Diagnostic Consensus Criteria. Based on whether or not they had BPD, the preterm infants were divided into groups for control or BPD. This work was evaluated and approved by the ethics committee of Mayo Institute of Medical Sciences. Faizabad Road.Barabanki. Lucknow ; 226010. Uttar Pradesh**.**

On days of life (DOL) 7, 14, 21, and 28, we collected 1.5 mL of spot urine samples from the preterm children in the morning. The samples were kept at -80°C until they were needed. The samples were defrosted at 20–26°C, and then centrifuged at 300 x g for 10 min at 4°C. Enzyme-linked immunosorbent assay kits were used to assess the amounts of 8-OHdG and NT-proBNP in urine and we additionally assessed urine creatinine (Cr) using spectrophotometry[15]. The findings were reported in terms of ng of NT-proBNP per mg of Cr and ng of 8-OHdG per mg of Cr, or the urine 8-OHdG/creatinine ratio (UDGCR), ng/mg Cr. Every sample was examined in triplicates .

The statistical analyses were conducted using SPSS (SPSS Inc., Chicago, IL, USA; version 24.0). T-tests are used to compare normally distributed continuous variables, which are shown as the mean ± standard deviations.

Table 1 Perinatal characteristics & Preterm infant characteristics

Perinatal characteristics			
Variable	**Control group (n = 43)**	**BPD group (n = 41)**	**P-value**
Maternal age	28.3 ± 5.2	32.4 ± 3.5	0.785
Cesarean (%)	72.9	63.4	0.698
PROM > 18h(%)	26.3	29.4	0.587
Antenatal steroid usage (%)	64.2	60.7	0.663
PIH (%)	39.4	45.2	0.591
GDM (%)	21.4	36.4	0.357
Chorioamnionitis (%)	27.4	41.6	0.042
Preterm infant characteristics			
GA (weeks)	31.1± 2.54	30.5 ± 2.65	<0.001
Birth weight (g)	1345.2 ± 198.2	1247.4 ± 146.2	<0.001
Male sex (,%)	41.2	58.8	0.261
Apgar score (1 min)	8.00 (6.00,10.00)	9.00 (6.00,10.00)	0.051
Apgar score (5 min)	9.00 (9.00,10.00)	9.00 (9.00,10.00)	0.587
Surfactant therapy (n, %)	58.1	65.3	0.469
Total hospital stay (day)	47±12	56±19	<0.001
Mechanical ventilation (day)	13±9	33 ±21	<0.001
Oxygen consumption duration (day)	21±15	50 ±29	<0.001

Table 2- Cutoff values of variables

Variable	**AUC**	**Cutoff (ng/mg Cr)**	**Sensitivity**	**Specificity**	**P-value**
A	0.622	17.	0.624	0.782	<0.001
B	0.714	20.9	0.781	0.889	<0.001
C	0.801	19.52	1	0.771	<0.001
D	0.902	17.21	0.764	0.891	<0.001
E	0.643	16.58	0.425	0.885	<0.001

F	0.757	12.47	0.567	0.897	<0.001
G	0.794	10.56	0.764	0.654	<0.001
H	0.899	8.87	1	0.715	<0.001

Note -A=UDGCR DOL 7,B= UDGCR DOL 14,C= UDGCR DOL 21 & D=UDGCR DOL 28
E=UNBCR DOL 7,F= UNBCR DOL 14,G= UNBCR DOL 21& H=UNBCR DOL 28

Results

198 preterm babies were enrolled, of whom 84 were included in the analysis (43 in the control group and 41 in the BPD group). There was no difference in the preterm infants' prenatal features between the two groups. In comparison to the control group, the BPD group had a younger GA, a lower birth weight, and an extended hospital stay overall. In addition, there were notable differences between the two groups' mechanical breathing duration, oxygen exposure time, retinopathy of prematurity (ROP), PDA, and pulmonary hypertension (PH) occurrences (Table 1).

From DOL 7 (17.56 vs. 16.58 ng/mg Cr, $P < 0.001$; Table 2) to DOL 28 (20.94 vs. 12.47 ng/mg Cr, $P < 0.001$; Table 2), the 8-OHdG level was considerably greater in the BPD group than in the control group. From DOL 7 (19.52 vs. 10.56 ng/mg Cr, $P < 0.001$;Table 1) to DOL 28 (17.21 vs. 8.87 ng/mg Cr, $P < 0.001$;Table 1), the NT-proBNP concentrations in the BPD group were also considerably greater than in the control group.

The sensitivity, specificity, and AUC for NT-proBNP on DOL 28 were 100.0%, 80.0%, and $P < 0.001$, respectively.

On DOL 7, 14, 21, and 28, there was a significant correlation ($P < 0.001$) between the urine 8-OHdG level and the NT-proBNP level. Additionally, it was demonstrated that levels of 8-OHdG and NT-proBNP were significantly correlated with the length of mechanical breathing and the amount of time spent exposed to oxygen from DOL 7 to 28 ($P < 0.05$;Table 1).

Discussion

For the first time, urine 8-OHdG and NT-proBNP levels are prospectively measured in very low and extremely low birth weight infants across time, and relationships with the development of BPD in the first 28 days of life are assessed. This study also aims to investigate the relationship between the levels of NT-proBNP and urine 8-OHdG. We observed substantially higher levels of NT-proBNP and 8-OHdG in the urine of preterm babies with BPD from DOL 7 to DOL 28 compared to those without BPD. Additionally, from DOL 7 to 28, there was a significant connection between the levels of NT-proBNP and 8-OHdG. Moreover, the levels of NT-proBNP (DOL 7-28) and 8-OHdG (DOL 14 to 28) were substantially correlated with BPD after controlling for confounding variables[15]. The best predictive cutoff values for urine 8-OHDG and NT-proBNP for BPD from DOL 7 to DOL 28 were also found using ROC curves. These findings might lead to early identification of preterm infants at high risk for BPD and the development of efficient preventative and therapeutic strategies[16&17].

RDS, PDA, BPD, ROP, necrotizing enterocolitis, intraventricular haemorrhage, and periventricular leukomalacia are among the illnesses caused by oxygen-free radicals. Furthermore, oxidative stress plays a crucial role in lung damage progression, starting with acute inflammatory injury (as in RDS) and ending with pulmonary microvascular remodelling and defective alveolarization, which ultimately results in BPD. DNA damage caused by endogenous oxidative stress is shown by the biomarker 8-OHdG[18]. After examining the connection between the 8-OHdG level in TA and BPD, researcher found that there was a significant ($P<0.05$) correlation between the TA 8-OHdG level and BPD on postnatal day[19 &20]. Furthermore, 8-OHdG is eliminated in the urine without undergoing further metabolic. Researchers discovered that compared to babies with no to mild BPD, preterm infants with moderate to severe BPD had greater urine levels of 8-OHdG[21]. Consequently, we postulated that 8-OHdG in urine might serve as a biological marker for non-invasive BPD prediction. significantly water-soluble. Our findings lend credence to this theory, It was shown that from DOL 7 to DOL 28, the BPD group's urine 8-OHdG level was considerably greater than that of the control group[22]. Additionally, our research revealed a favourable correlation between the duration of mechanical breathing and the amount of time spent exposed to oxygen on DOL 7–28 and the urine 8–OHdG level[1,4 6 23]. Most notably, Serum NT-proBNP levels have been shown in further research to be a useful biomarker for the prediction, diagnosis, and treatment of respiratory disorders, including BPD. Moreover, correlations have been shown in recent research between urine NT-proBNP concentrations and problems associated with preterm delivery, including ROP, PDA, and PH[24,25&26].

Our prospective analysis showed that preterm children who had BPD several weeks later had substantially higher urine NT-proBNP concentrations from DOL 7 to 28 ($P < 0.05$). This result was similar with trends in serum NT-proBNP level reports by other researchers, and it related circulatory stress to BPD in the first four weeks of life for preterm children[8 &12]. Additionally, we discovered a favourable correlation between the length of mechanical ventilation and the amount of time spent exposed to oxygen and the urine NT-proBNP levels from DOL 7 to 28. Pulmonary microvascular dysplasia and delayed alveolar development are hallmarks of BPD. By altering vascular tone, perinatal exposure to oxidative stress, postnatal hyperoxia, and extended mechanical breathing may exacerbate the damage to pulmonary vascularization[15 &19]. Increased pulmonary vascular pressure and diastolic dysfunction may be associated with elevated serum NT-proBNP levels. In urine samples collected from DOL 7 to 28, we found a consistently significant connection ($P < 0.001$) between

NT-proBNP levels and an oxidative stress-related biomarker, 8-OHdG. Thus, we postulated that oxidative stress may be linked to elevated NT-proBNP levels in preterm babies with BPD. The plasma NT-proBNP level at DOL 28 exhibited a modest prognostic value for BPD severity, according to a prospective observational research.

Conclusion

The results of our investigation demonstrated a continuous positive correlation between NT-proBNP levels and urine 8-OHdG from DOL 7 to 28. Early BPD identification and treatment might be improved by the discovery of practical and trustworthy biomarkers to identify high-risk babies.

Conflict of interest

The research was carried out without any financial or commercial ties that may be seen as having a conflict of interest, the authors disclose.

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