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URINARY INFLAMMATION AND OXIDATIVE STRESS INDICATORS IN VERY LOW BIRTH WEIGHT INFANTS IN RELATION WITH THE DEVELOPMENT OF BRONCHOPULMONARY DYSPLASIA

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Abstract
Bronchopulmonary dysplasia (BPD) now affects preterm newborns nearly exclusively. Apart from preterm, the pathogenesis can also be influenced by other variables like as inflammation and oxygen toxicity. Urinary inflammatory and oxidative stress markers were compared between the groups with no or mild BPD and those with moderate to severe BPD, as well as between BPD cases with significant early lung disease such as respiratory distress syndrome (RDS) (referred to as "classic" BPD) and those with minimal early lung disease (referred to as "atypical" BPD). There were sixty patients in all, with a gestational age of thirty weeks and a birth weight of twelve hundred fifty grammes. During the first, third, and seventh days of life, urine samples were collected to assess the levels of 8-hydroxydeoxyguanosine (8-OHdG). The 8-OHdG levels and the length of mechanical ventilation exhibited a significant connection on the third day.
The independent risk factor for the development of moderate/severe BPD was the 8-OHdG levels on the 7th hour. The third-day 8-OHdG levels in "classic" BPD were greater than those in "atypical" BPD. The LTE 4 readings on the seventh day were greater in "atypical" BPD than they were in "classic" BPD. According to these findings, oxidative DNA damage may play a major role in the pathophysiology of contemporary BPD, whereas the continuous inflammatory process may play a significant role in "atypical" BPD.

Keywords: Chronic lung disease , Leukotrienes , 8-hydroxydeoxyguanosine , pre-term infants , systematic inflammatory response

Introduction

Bronchopulmonary dysplasia (BPD), the most common chronic lung disease in newborns, presents clinically as severe fibrosis or atelectasis with cystic changes and necessitates mechanical ventilation. BPD is preceded by respiratory distress syndrome (RDS)[1]. In this era of surfactant and prenatal steroids, there is a decreased incidence of severe RDS, although BPD still occurs despite the use of moderate mechanical breathing methods[2]. According to patterns of oxygen dependency, multiple reports have demonstrated the heterogeneity of current BPD. BPD characterised by minimal early lung disease, also known as the "atypical" or "new" form of BPD, is becoming more common in the population of very low birth weight infants (VLBWI) compared to the classical type of BPD, which is distinguished by initially significant lung disease[3]. Numerous investigations have revealed a connection between the pathophysiology of contemporary BPD and prenatal traumas, including dysregulation of pro- and antiinflammatory cytokines, the intrauterine immune response, and maternal chorioamnionitis[4]. Additionally, we showed in a prior study that umbilical cord blood from infants with BPD who had minimal early lung disease had higher levels of plasma KL-6, a specific lung injury marker[5]. This suggests that lung injury occurs during the prenatal period in infants who go on to develop current BPD. Furthermore, because preterm babies' immature antioxidant systems make them particularly susceptible to reactive oxygen species, oxidative metabolites have been suggested to play a significant role in the pathophysiology of BPD[6]. Using the "double-hit" animal model, we recently revealed that oxidative stress and the inhalation response were both important factors in the pathophysiology of BPD[7].
There is ongoing discussion over the precise processes and the relative contributions of each element to the pathophysiology of contemporary BPD in the post-surfactant age[8].
The urine markers 8-hydroxydeoxyguanosine (8-OHdG) and leukotriene E4 (LTE4), which, as previously noted, reflect the two pathways of oxidative damage and inflammation, respectively, in connection to the eventual development of present BPD, were assessed in this investigation[9]. LTE4 is an inflammatory marker that may be found in urine and is linked to the severity of bronchial asthma. It is a recognised consequence of the breakdown of the phospholipid bilayer of the cell membrane. The molecule 8-OHdG is a marker of oxidative DNA damage; in the context of oxidative stress

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during DNA synthesis, oxidised 8-hydroxyguanine (8-OHGua) is incorporated into DNA instead of a regular guanine base and is broken down by endonuclease to produce 8-OHdG, which is detectable in urine[10]. By measuring these urine biomarkers in the first week of life, we also aimed to determine whether there are different underlying mechanisms in the pathogenesis of BPD according to their clinical presentations: BPD with significant early lung disease (also known as "classic" BPD) as compared to BPD with minimal early lung disease (also known as "atypical" BPD).

Materials and methods

Included were preterm infants hospitalised to Mayo Institute of Medical Sciences. Faizabad Road.Barabanki. Lucknow ; 226010. Uttar Pradesh. India in neonatal critical care unit between July 20015 and June 2017, with a gestational age of 30 weeks or a birth weight of 1250 g[11]. We did not include infants who died before they reached a corrected age of 36 weeks, or who had significant congenital deformities or chromosomal abnormalities. Doctors/ residents had examined the clinical features of the patients and their moms in the medical records. Our institutional local research ethics committee authorised the study methodology, and parents gave their informed consent before any urine samples were taken.

Methods [12]

The National Institute of Child Health Workshop (NICHD) definitions and criteria were used to characterise bronchopulmonary dysplasia and its severity. The criteria or guidelines as per NICHD were used to subclassify the BPD group into two groups: "classic" BPD was defined as cases with respiratory distress syndrome (RDS) that did not resolve and required continuous oxygen supplementation for at least 28 days; "atypical" BPD was diagnosed in cases of BPD without RDS or in cases of BPD that were preceded by initial RDS that resolved within 10 days and did not require oxygen supplementation for at least 72 hours, in addition to the 28-day oxygen requirement. When there was acute respiratory distress, a growing oxygen need, and radiologic findings consistent with RDS, RDS was diagnosed. Each baby diagnosed with RDS was given up to three doses of surfactant replacement treatment until they demonstrated clinical improvement.

When at least one positive blood culture result was combined with clinical symptoms such as hypotension or left-shifted white blood cell counts with immature to total WBC ratios greater than 0.2, sepsis was confirmed. The diagnosis of patent ductus arteriosus (PDA) was limited to cases when echocardiography revealed symptoms of PDA[13]. A body temperature of 37.8°C or higher and two or more of the following symptoms were considered indicative of clinical chorioamnionitis (cCAM): uterine pain, malodorous vaginal discharge, maternal leukocytosis (> 15 000/mm3), maternal tachycardia (> 100/min), or foetal tachycardia (> 160/min). The existence of acute inflammatory alterations in the membrane and/or the chorionic plate was described as histologic chorioamnionitis (hCAM)[14]. Using the updated Bell's criteria, necrotizing enterocolitis (NEC) was identified at stages equivalent to or greater than II. A germinal matrix/intraventricular haemorrhage with grades equivalent to or greater than grade II verified by ultrasonographic diagnostics was described as an intraventricular haemorrhage (IVH) by Volpe. The following formula was used to determine the total quantity of oxygen supplementation given for the first three and six days of life: total extra oxygen supplementation = supplemented extra oxygen concentration (%) (fraction of inspired oxygen-21)Xtime(h)[14].

Table 1 Clinical characteristics of groups

	Moderate/severe BPD (n = 48)	No/mild BPD (n = 32)
Maternal age (y)	35.5±4.3	36.2±3.2
Gestational age (wk)*	27.5±2.7	29.4±3.5
Birth weight (g)*	950 [610-1250]	1054 [780-1380]
1-min Apgar score	3 [0-7]	4 [0-7]
5-min Apgar score*	4[1-8]	8 [2-10]
Caesarean section,(%)	80.2	84.4
PROM > 18 h, (%)	41.9	29.5
cCAM, (%)	11	NA
hCAM, (%)	54.8	45.4
Antenatal steroid, (%)	92.4	84.5
Postnatal steroid, (%)	19.4	NA
RDS, (%)	73.3	29.3
Duration of O_2 (d)*	85.2	11.2
Duration of MV (d)*	15.2	3.5
PDA, (%)	89.2	78.1
Sepsis, (%)*	47.1	19.8
NEC, (%)*	21.1	NA
IVH > Gr2, (%)	11.2	NA
ROP, n(%)	63.1	25.1
Hospital stay (d)*	94.2±25.1	57.7±31.2

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Table 2. Correlation between the duration of mechanical ventilation and marker level.

Time point	Correlation coefficient
Day 1 LTE_4	0.145
Day 3 LTE_4	0.0001
Day 7 LTE_4	0.002
Day 1 8-OHdG	0.019
Day 3 8-OHdG	0.201*
Day 7 8-OHdG	0.012

* *P*-values lower than 0.05 were considered statistically significant.

Table 3 Clinical characteristics in the classic and atypical BPD groups

	Classic BPD (n = 17)	Atypical BPD (n = 18)
Maternal age (y)	35.2±2.9	36.3±2.5
Gestational age (wk)	27.4±2.1	29.3±4.1
Birth weight (g)	845.0 [510.0-1180.0]	980.0 [470.0-1350.0]
1-min Apgar score,	2 [0-5]	3 [0-6]
5-min Apgar score,	4 [1-6]	5 [0-6]
Caesarean section, (%)	84.2	71.8
PROM > 18 h, (%)	30.5	45.2
CCAM, (%)	10.5	15.5
HCAM, (%)	45	60
Antenatal steroid, (%)	80	100
Postnatal steroid, (%)	25	10
RDS, (%)*	100	10
Duration of O_2 (d)	81.2±25.2	55.2±26.4
Duration of MV (d)*	26.5±2.4	7.5±5.2
Sepsis, (%)	75	50
Cord IgM (mg/dL)	8.5 ±6.2	11.5±6.4
Cord CRP (mg/dL)	0.07	0.05
Day 7 CRP (mg/dL)	0.11	0.08
Hospital stay (d)	101.9±54.6	89.3±65.8

Values are expressed as medians [ranges], *$p < 0.05$.

Assay 8-OHdG[15]

Day 1 pee samples were collected within 24 hours of delivery, and day 3 and 7 morning spot urine samples were kept in polypropylene tubes at —20°C until analysis. Following defrosting, the specimens underwent a 5-minute, 10,000-g centrifugation at 4°C. An ELISA was used to assess the levels of urinary LTE4, and a highly sensitive ELISA kit was used to detect the levels of 8-OHdG. Urine creatinine (Cr) was measured using an ELISA machine in order to standardise the data. The results were reported as picograms of LTE4 and nanograms of 8-OHdG/milligram of Cr.

Statistics

Every sample was examined twice. conducted using the Fisher's exact tests for categorical data and the Mann-Whitney U-test, which was also utilised for continuous variables. P-values were deemed statistically significant if they were less than 0.05.

Results

After completing the requirements for inclusion where out of selected newborns, one patient had Down syndrome and two died before 32 weeks of corrected age. Out of the 83 patients who were left, 80 had at least one sample that was examined at three different time points—the first, third, and seventh days of life. The average birth weight was 950 to 1054 g, and the average gestational age was 27 to 29 weeks. The mean duration of hospitalisation was 57 to 94 days. Out of the 80 patients whose samples were examined, 35 had a diagnosis of BPD; 17 patients had a diagnosis of "classic" BPD, and 18 patients had a diagnosis of "atypical" BPD.

There was a statistically significant lowering trend in the median LTE4 levels of the study population on days 1, 3, and 7, with values of 201.1, 124.2, and 78.1 pg/mg Cr, respectively.

LTE4 values were 514.4, 1040.4, and 76.9 pg/mg Cr for the no/mild BPD group and 240.0, 187.4, and 90.0 pg/mg Cr for the moderate/severe BPD group, respectively.

The median 8-OHdG values on days 1, 3, and 7 were, respectively, 1.5, 2.8, and 1.8 ng/mg Cr in the group with no or mild BPD and 1.4, 2.8, and 3.2 ng/mg Cr in the group with moderate to severe BPD. On day 7, there was a statistically significant difference ($p = 0.002$) between the two groups . Factors such as gestational age, birth weight, 5-min Apgar score, sepsis, length of mechanical ventilation, and necrotizing enterocolitis were included that demonstrated differences with a p-value of 0.1 between the moderate/severe BPD group and the non/mild BPD group.

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Urinary 8-OHdG level in "classic" BPD was 1.4 ng/mg Cr on day 1, and it rose considerably to 2.4 ng/mg Cr on day 3 ($p = 0.023$) and 2.1ng/mg Cr on day 7. On day 3, the urinary 8-OHdG levels were significantly higher in "classic" BPD than in "atypical" BPD ($p = 0.038$). A cut-off value of 2.20 pg/mg Cr showed sensitivity of 84.5%, specificity of 59.9%, and receiver operating characteristic curve analysis of urinary 8-OHdG on the 3rd day of life in the identification of classic BPD. The sensitivity and specificity for 8-OHdG on the 3rd day of life for predicting "classic BPD" over atypical BPD were also significant.

Discussion

A multitude of causes, including infection, oxidative stress, and baro/volutrauma, can harm developing lungs and lead to the development of BPD. In order to improve our understanding of the pathophysiology of BPD in babies and to aid in early identification, several biomarkers in blood, tracheal aspirate (TA), and even urine have been examined. The analysis of biomarkers in the TA may be appropriate for the direct assessment of juvenile lung pathophysiology; however, the invasive approach used to extract the TA has drawbacks, including challenges with universal sampling and normalisation. The non-invasive nature of urine sample eliminates the possibility of anaemia from excessive blood sampling[1, 15]. Urinary biomarker analysis has increased in pre-term infants with various conditions since a recent study found a correlation between increased levels of urinary bombesin-like peptide and an increased risk of developing BPD [16]. However, the number of published studies on this topic is relatively small. Two urine markers that represent the processes of oxidative damage and inflammation, 8-OHdG, were assessed in the current investigation in relation to the eventual development of BPD[17]. We also looked into the possibility that the pathophysiology of BPD differs depending on how the disorder manifests clinically. As of yet, there is no accepted theory for BPD subclassification based on pathophysiology or clinical presentation. Nonetheless, a few investigations raised the prospect that variations in the aetiology might have an impact on how BPD presents clinically[18]. We thus examined the levels of urine markers in BPD patients with low early lung disease (referred to as "atypical" BPD) and those with severe early lung disease (known as "classic" BPD).

Because our study population had a relatively smaller gestational age and birth weight than previous studies, the incidence of BPD (70%) was slightly higher than other reports (~ 51%) and similar to our previous data (67%), which included pre-term infants who were 13 weeks gestational age. The median gestational age and birth weight in the group with moderate/severe BPD were much lower than those in the group with no/mild BPD. In order to compare the groups, we used logistic regression analysis, which included variables like gestational age, birth weight, and 5-min Apgar scores to account for these established risk factors. Lower gestational age and birth weight are the most significant risk factors for the development of BPD[19].

Exposure to excessive oxygen concentrations has been recognised as an independent risk factor in the pathophysiology of BPD since Northway's initial report[20]. A prior evaluation of the data on the contribution of oxidative stress to the development of BPD, based on measures of oxidative stress indicators, was conducted. The term "oxidative stress" is now used more broadly to refer to an imbalance between oxidant and antioxidant forces as well as excessive oxygen supplementation[21]. Urinary 8-OHdG is one of the numerous oxidative stress indicators that may be collected using non-invasive sampling procedures. This is why we selected it. There are currently very few research examining 8-OHdG levels in the urine of preterm populations. In comparison to term controls, researchers found that VLBW newborns had substantially greater levels of 8-OHdG, and pre-term infants less than 36 weeks of corrected age had higher levels of 8-OHdG than older infants. Our research is also the first to show a relationship between the length of time a patient is dependent on a mechanical ventilator, a measure of oxidative stress determined by 8-OHdG on the third day of birth, and the clinical severity of BPD. These findings imply that urine 8-OHdG may serve as a useful predictor of the emergence of relatively severe BPD later on, and that oxidative DNA damage remains a significant risk factor for BPD even in the post-surfactant era[22]. According to a recent study, preterm newborns with retinopathy of prematurity (ROP) had higher amounts of 8-OHdG in their leukocytes and urine than infants without the condition. It is well recognised that ROP and BPD are oxidative stress disorders in preterm newborns, and that oxidative stress-induced DNA damage can lead to maldevelopment of organs in extremely preterm children. Additionally, throughout the first week of life, we looked at the dynamic pattern of urine 8-OHdG levels in the "classic" and "atypical" BPD groups. In the "classic" BPD group, the 8-OHdG level increased considerably on day three compared to day one; however, in the "atypical" BPD group, there was no significant change throughout the first week of life. This might have happened as a result of the 'traditional' BPD group receiving more intensive respiratory care due to their considerable early lung illness, such as RDS. Furthermore, the findings raise the prospect that oxidative stress may not be the only mechanism involved in the emergence of "atypical" BPD.

When we separated the "atypical" BPD group based on the severity of BPD, we also discovered that on day 7, the levels of LTE4 were much greater in the severe BPD group than in the mild/moderate BPD group (data not shown). These results imply that, despite limited early lung illness and less mechanical and oxidative lung injury, there may have been a prolonged inflammatory insult in this group throughout the first week of life. Nonetheless, there were no noteworthy variations in the rates of sepsis, NEC, and maternal CAM between the "atypical" and "classic" groups, indicating that the underlying mechanism of the prolonged inflammatory process is still unknown[24]. To fully understand how the inflammatory process contributes to the development of "atypical" BPD, more research is required.

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CAM has been linked to the development of BPD, according to previous research; nevertheless, some papers have found no linkage at all or negative correlations between hCAM and BPD. The incidence of cCAM and hCAM did not significantly differ in the current study between the groups with no or mild BPD and those with moderate to severe BPD, nor between the "classic" and "atypical" BPD categories[25]. On the first day of life, however, there was a marginally significant ($p = 0.052$) rise in 8-OHdG levels in the population with maternal hCAM compared to the non-hCAM population (data not shown). This raises the idea that newborns whose mothers have hCAM may be more vulnerable to oxidative stress.

Conclusion

Day 7: The degree of oxidative stress indicated by 8-OHdG levels is a separate risk factor for the development of moderate or severe BPD, and the length of mechanical ventilation was significantly correlated with the levels of 8-OHdG on the third day. According to these findings, urine 8-OHdG may serve as a useful predictor of the ensuing onset of BPD, and even in cases of BPD that are currently receiving little respiratory treatment, its severity and oxidative DNA damage remain significant risk factors. However, even though there was no RDS and very little mechanical ventilation throughout the early newborn period in this group, the persistently elevated urine LTE4 levels on the seventh day, which were exclusively observed in "atypical" BPD, point to a prolonged inflammatory response. These results could point to variation in the contemporary era's pathophysiology and clinical course of borderline personality disorder.

Note Values are expressed as medians [ra nges], $*p < 0.05$.
BPD, bronchopulmonary dysplasia;
PROM, premature rupture of membrane;
CCAM, clinical chorioamniionitis;
HCAM, histological chorioamnionitis;
MV, mechanical ventilation;
RDS, respiratory distress syndrome;
PDA, patent ductus arteriosus;
NEC, necrotizing enterocolitis;
IVH, intraventricular haemorrhage;
ROP, retinopathy or prematurity.

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