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# Evaluation of treatment with hydrocortisone on oxidant/antioxidant system in preterm infants with BPD

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**Abstract. – AIM: Evidence that oxidative stress plays a role in the development of bronchopulmonary dysplasia (BPD). There is a close relationship between oxidative stress and inflammation. In this study, it is aimed to investigate influences of hydrocortisone used in the treatment of BPD on anti-oxidant system in preterm infants with BPD.**

**PATIENTS AND METHODS: The study enrolled 33 infants with severe BPD who were undergone inpatient treatment in neonatal intensive care unit (NICU) of our Hospital and received therapy with hydrocortisone. Total oxidant status (TOS) and total anti-oxidant capacity (TAC) levels of infants enrolled to the study before and one week after the hydrocortisone therapy were studies and oxidative stress index levels were calculated. Pre- and post-treatment TOS, TAC and OSI index levels were statistically compared.**

**RESULTS: In preterm infants with BPD, who were enrolled into the study, TOS and OSI index were found high, whereas TAC values were low. Following the treatment with hydrocortisone, statistically significant decrease in TOS and OSI index and statistically significant elevation in TAC levels were found in comparison with pre-treatment levels.**

**CONCLUSIONS: The treatment with hydrocortisone, which is used for BPD, improves anti-oxidant system and reduces oxidative stress in infants with BPD. There is need for further studies in order to clarify the physio-pathogenesis.**

*Key Words:*
Bronchopulmonary dysplasia, Hydrocortisone, Oxidant/antioxidant system.

## Introduction

Bronchopulmonary dysplasia (BPD) is a frequent complication of premature newborns born at less than 28 weeks of gestation[1]. While the origins of this disorder are multi-factorial, these diverse pathologies are linked by oxidative stress, a major causative factor[2] Although necessary to sustain life, oxygen therapy directly exposes the lung to high concentrations of inspired oxygen, increasing the burden of toxic reactive oxygen species (ROS). Respiratory bursts by inflammatory cells and normal mitochondrial respiration also contribute to the oxidative stress. In healthy tissues, free radical scavengers and antioxidant systems interrupt the cycle of oxidant-induced tissue injury. However, the antioxidant capacity is limited in the premature newborn making the increased oxidative burden and risk for tissue injury increasingly significant. Given the limited antioxidant capacity of the premature newborn, strategies to limit the oxidative burden become critical for this vulnerable population[2].

A close link between inflammation and oxidative stress has been established[1]. Since inflammation plays a prominent role in pathogenesis of BPD, intravenous and oral steroids have been used as anti-inflammatory agents to alter the course of lung disease in premature infants. In this study, we aimed to investigate influence of postnatal hydrocortisone therapy on anti-oxidant system of infants with BPD.

## Patients and Methods

This prospective pilot study was conducted in a tertiary referral neonatal intensive care unit (NICU) placed in Ankara Zekai Tahir Burak Maternity Teaching Hospital. The study was approved by the local Research Ethics Committee. Between January 2009 and August 2010 infants with BPD were recruited to this study. Infants were excluded if they had a diagnosis of suspected or proven infection, significant congenital malformation, and clinical evidence of PDA, NEC and intestinal hemorrhage or perforation.

Patient characteristics (gestational week, birth weight, gender, type of delivery etc.), maternal, prenatal and postnatal features were all recorded.



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Preterm babies (< 28 week gestational age or < 1000 g birth weight) who were ventilator dependent approximately at or beyond 3 weeks of age (defined as rescue treatment) or were oxygen dependent on postmenstrual 36th week without evidence of any infection (defined as BPD treatment) were enrolled in the study. Hydrocortisone was used orally in an initial dose of 1 mg/kg twice a daily for a week and then the dose was tapered by 10-20% every other day regarding to clinical response. Clinical response was defined as reduced supplemental oxygen requirement (< 30%) and/or weaning from respiratory support (ventilation/n CPAP). Plasma samples were obtained for TAC and TOS before the treatment with hydrocortisone and following 1-week complete dose cycle and OSI indexes were calculated.

### Biochemical Methods

Venous blood samples were placed in straight tubes containing EDTA. The samples were centrifuged for 10 minutes in plastic tubes at a speed of 3500 rpm. The supernatant was stored at –80° C until the TAC and TOS were evaluated. All plasma samples were studied on the same day.

The TAC was measured using Erel's TAC method[4], which is based on the bleaching of the characteristic colour of the relatively stable 2,2'-azino-bis (3-ethylbenz-thiazoline-6-sulfonic acid) (ATBS) radical cation by antioxidants. The results were expressed in mmol Trolox equiv/L. The serum thiol (the total number of SH groups) content was measured using 5,5-dithiobis-2-nitrobenzoic acid (DTNB). The plasma TOS was measured using Erel's TOS method[5,6], which is based on the oxidation of ferrous ion to ferric ion in the presence of various oxidative species in acidic medium and the measurement of the ferric ion using xylenol orange. The results were expressed in μmol $H_2O_2$/L. Erel's TAC and TOS methods are colorimetric and automated, and the precision of the assays is excellent, The OSI was the ratio of TOS to TAC. To calculate the OSI, the units of TAC (mmol Trolox equivalent/L) were changed to μmol Trolox equivalent/L, and the OSI value was calculated as OSI = [(TOS (μmol /L))/(TAC (μmol/L))/100].

### Statistical Analysis

The data are presented as the mean ± standard deviation, frequency, or percentage. Paired-samples *t*-tests and independent-samples *t*-tests were used for continuous variables; the chi square test was used for categorical variables. $p < 0.05$ was considered statistically significant. The statistical analyses were performed with SPSS 17.0 for Windows (SPSS Inc., Chicago, IL, USA).

## Results

Thirty three[33] infants with BPD, who were receiving treatment with hydrocortisone, were included in the study. Demographics and clinical characteristics of patients are given in Table I. In infants with BPD, who were enrolled into the study, it was found that TOS and OSI index after the treatment with hydrocortisone were significantly lower than TOS and OSI values before the treatment with hydrocortisone ($p < 0.0001$). A statistically significant elevation was found in post-steroid TAC values ($p = 0.005$) (Table II).

**Table I.** Demographic and clinical characteristics of patients.

| | |
|---|---|
| Gestational age; week, mean ± SD | 26.7 ± 2.3 |
| Birth weight; g, mean ± SD | 977 ± 271 |
| Maternal age, y, mean ± SD | 27 ± 4.3 |
| Gender; (F/M) | 12/21 |
| Respiratory distress syndrome (RDS); %, (n) | 75.8% (25) |
| Necrotizing enterocolitis (NEC); %, (n) | 6.1% (2) |
| Retinopathy of prematurity (ROP) (≥ evre3); %, (n) | 27.3 (9) |
| Intracranial hemorrhagia (≥ Grade 3); %, (n) | 6.06% (2) |
| Patent ductus arteriosus (PDA); %, (n) | 60.6% (20) |
| Mean hydrocortisone initiation time; day, mean ± SD | 27.8 ± 6.5 |
| Hydrocortisone using time; day, mean ± SD | 23.3 ± 14.4 |
| Caffeine; %, (n) | 90.9 (30) |
| Diuretics; %, (n) | 72.7% (24) |
| Ibuprofen; %, (n) | 36.4% (12) |

## Discussion

Evidence that oxidative stress plays a role in the development of chronic lung disease (CLD) has accumulated over the last 7-8 yr[7] Preterm infants are often exposed to increased oxidative stress due to exposure to high oxygen concentrations in combination with low surfactant concentrations, lowered antioxidant defenses, and decreased ability to induce antioxidant enzymes[8]. Immaturity per se, inadequate nutrition, inflammation and how the baby is ventilated also add to the total oxidative stres which might trigger changes leading to permanent lung damage. A further insight was gained when oxidative stres and inflammation were linked and it was understood that these two phenomena could be two sides of the same coin. During the last years it has been better understood that oxidative stress may activate transcription factors which may transcribe genes that are related to inflammation, apoptosis, as well as oxidative stress.

Inflammatory cells, in particular neutrophils, are involved in the pathogenesis of bronchopulmonary dysplasia (BPD)[9]. The inflammatory response is triggered by proinflammatory cytokines, lipid mediators, and complement activation[10]. Additionally, increased protein carbonyls in tracheal aspirates of preterm babies have been shown to correlate with myeloperoxidase activity from neutrophils[11]. An imbalance between proteases and anti-protease activity in the respiratory tract has been reported in neonates with BPD[12], which may lead to further lung injury and abnormal remodeling.

More recently, Vento et al[13] reported significant correlations between inspired oxygen concentrations and total antioxidant capacity or uric acid concentrations in tracheal aspirates of preterm babies. The lungs of premature infants are particularly sensitive to the injurious effect of oxygen and mechanical ventilation. The hypothesis of this study is that oxidative stress may decrease antioxidant levels in the lung, leading to higher concentrations of oxidized proteins in the epithelial lining fluid.

Clerch et al[14] showed that infection might aggravate the oxidative stress. This is in line with clinical experience that an infection superimposed on immature lungs contributes to the development of BPD. The study by Merritt et al[15] showing involvement of inflammatory cells in BPD was of great importance. Further, reoxygenation injury is associated with activation of leukocytes and endotelial cells. There is no principal difference between the inflammatory reaction evoked by microbes or by oxidative stress.

Kojima et al[16] found increased concentrations of the soluble form of intercellular adhesion molecule-1 (ICAM-1) in tracheal aspirates of infants with BPD. This molecule is upregulated on endothelial cells in response to endotoxin and cytokine stimulation and facilitates extravasation of neutrophils through the vessel wall. These cells are activated by cytokines and other mediators to produce oxygen radicals during hypoxia and reoxygenation. Furher, increased concentrations of soluble IL-2 receptor have been found in sera from patients with BPD. This is a marker of T cell activation and may indicate an early sign of transition from inflammation to fibrosis.

A main effect of postnatal steroids in preventing or improving BPD is probably mediated via decreasing the oxidative stres by reducing the inflammatory responce. Although there are studies addressing the relationship between antenatal steroids and anti-oxidant activity in preterm infants, there is no study examining postnatal steroids and anti-oxidant activity.

Vento M et al[17] was found an association between antenatal steroids and antioxidant activity and postnatal oxidative stress. In a prospective cohort study, extremely preterm neonates receiving antenatal steroids (CORT) or not (NOCORT) were enrolled. An association between antenatal steroids and activities of antioxidant enzymes and glutathione cycle enzymes in cord blood was found. In addition, reduced oxidative stress Antenatal steroids are accompanied by a reduction in postnatal oxidative-stress-derived conditions and

**Table II.** TOS, TAC and OSI measurements of patients.

| | Before treatment | After treatment | *p* |
|---|---|---|---|
| TOS, (μmol $H_2O_2$/L) | 46.7 ± 3.17 | 21.1 ± 4.82 | < 0.0001 |
| TAC, (mmol Trolox equiv/L) | 1.74 ± 0.1 | 2.18 ± 1.6 | 0.005 |
| OSI, (Arbitrary Unit) | 0.13 ± 0.04 | 0.08 ± 0.02 | < 0.0001 |

increased antioxidant enzyme activity. Frank[18] showed that prenatal dexamethasone treatment was accelerated the maturation of both the surfactant system and the fetal lung antioxidant enzyme system. Findings of these studies provide support to our study.

## Conclusions

In the current study, we evaluated pre- and post-treatment total oxidant status and total antioxidant capacity levels in infants with BPD who received treatment with hydrocortisone. We found a statistically significant reduction in the post-steroid total oxidant status and a statistically significant elevation in anti-oxidant capacity. This finding can be explained via improvement in oxidative mechanisms via anti-inflammatory actions of steroids. However, further studies are needed in order to clarify influences of steroids on oxidative system.

### Conflict of Interest

The Authors declare that they have no conflict of interests.

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