Micronutrition and Enamel Disturbances in Bronchopulmonary Dysplasia

THESIS

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By

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Abstract

Bronchopulmonary Dysplasia (BPD) is a morbidity commonly associated with prematurity. BPD is a result of chronic requirement for positive pressure oxygenation due to incomplete or delayed pulmonary development. Patients with BPD are often subject to strict total parenteral (TPN) and micro nutrition requirements, as well as often requiring intubation for additional airway support. Limited studies to date have assessed the potential association between micro nutrition and disturbances in dental development. Subjects were recruited from the NCH BPD clinic. Birth variables as well as micro nutrition were assessed from the electronic medical record (EMR). All subjects received an oral exam to assess for caries status, hypoplasia, hypomineralization and any other dento-alveolar disturbances. Fluoride application, as well as anticipatory guidance with intent to establish an early dental home was completed. Data have been collected from 30 subjects with an overall mean age of 26 months, and a mean gestational age of 25 weeks. Out of the total cohort, 26% presented with a positive finding of enamel hypoplasia. There were no statistically significant differences between the hypoplasia (+) and (-) groups with respect to; number of Lasix IV or oral doses, number of therapeutic sucrose doses, presence of gastroesophageal reflux, documented history of necrotizing enterocolitis (NEC), or gestational age. There was also no difference in incidence/duration of intubation. Conclusion: Gross parameters of micro-nutrition were not directly associated with dental disturbances in children with BPD.
Dedication

This document is dedicated to my family.
Acknowledgments

I would like to express my genuine appreciation for all the guidance and patience of my advisor Dr. Thikkurissy and the rest of my committee.
Vita

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Fields of Study

Major Field: Dentistry
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Introduction

Prematurity

The National Institute of Child Health and Human Development (NICHD) defines prematurity as labor that begins before 37 weeks of gestation. One out of every 10 live births in the United States has been classified as preterm births.\(^1\) There are several underlying etiologies for prematurity. Prematurity can be a result of preterm ruptured membranes, preeclampsia, fetal distress, placenta previa, and intrauterine growth restriction as well as others.\(^2\) Female gender, exposure to antenatal steroids, and single birth as opposed to multiple births demonstrated more favorable outcomes in premature infants.\(^1\)

Survivability of preterm infants has been shown to correlate with gestational age. The survival rates range from about 50 percent at 23 weeks gestation to 93 percent and better at 26 weeks gestation and beyond.\(^3\) In another study in 2002, the first year survival rate for extremely low birth weight infants was 13.8% for infants with a birth weight of less than 500 g, 51% for infants with a birth weight of 500-749 g, and 84.5% for infants with a birth weight of 750-1000 g.\(^1\)
Morbidity Associated with Prematurity

Infants who once were too premature to survive are now experiencing longer lifespans with the help of new medical technology. A slow but steady decline in infant mortality has been reported in the United States. However, with this decrease in mortality rates there has come a corresponding come an increase in morbidity. Long term morbidities include; chronic lung disease, patent ductus arteriosus, intestinal issues associated with necrotizing enterocolitis, intraventricular hemorrhage resulting in hydrocephalus and brain damage, retinopathy, hearing impairment, periventricular leukomalacia causing cerebral palsy, as well as other cognitive and neurodevelopment disorders.

Approximately 8.3% of all births are classified as low birth weight. Low birth weight is classified as being of less than 2500 grams or 5.5 pounds. Infants with a birth weight of 1500 to 1000 grams are considered to have very low birth weight and less than 1000 grams is defined as extremely low birth weight. Every 100 grams added to birth weight increases the probability of favorable outcomes.

Immediate concerns associated with prematurity with low birth weight stem from underdeveloped organ systems and body regulations. This can cause breathing problems, infections, cerebral palsy, and developmental disabilities.

Airway Morbidity

The respiratory system is among the last organ systems to mature during gestation. Infants born prematurely may present with accompanying respiratory distress
syndrome (RDS), the leading cause of death in premature infants. About ten percent of premature infants develop respiratory distress syndrome in the United States. (NHLBI) These infants often lack an essential surface emulsifier called surfactant.\(^5\) Surfactant is a liquid complex of phospholipids and proteins that coat the inside of lungs to allow easy recoil and inhibit collapse. Without surfactant, there is increased respiratory effort by the infant and often cannot gain adequate gas exchange to support body systems. RDS is routinely treated with artificial surfactant. An infant may also require additional oxygen therapy in the form of a NCPAP (nasal continuous positive airway pressure) or a ventilator for adequate oxygenation.\(^5\)

Most infants improve within a few weeks of oxygen therapy and need supplemental oxygen less and less. Yet some do not improve quickly or get worse and RDS persists. These infants are diagnosed with a respiratory pathology called bronchopulmonary dyplasia (BPD).\(^1\)

**Therapies in Management of Premature Infants**

Healthcare providers face many challenges to from the moment a premature infant with low weight is born. Chief among these initially is thermoregulation, particularly in the immediate post-natal period. Hypothermia (<35\(^\circ\)C) can result in hypoglycemia, apnea, and metabolic acidosis. Preterm infants are prone to heat loss due to the poor barrier provided by their thin, poorly keratinized skin and decreased brown fat stores that usually are deposited in the third trimester of pregnancy. Temperature control is essential to early survival in and avoiding chronic oxygen dependency. The infant is
typically placed in a double walled incubator or radiant warmers maintain body temperature. Transport to the neonatal intensive care unit from the delivery room is carried out using either warmed blankets or cellophane wrap.\textsuperscript{1}

Another pressing concern in the immediate post-natal period is hypoglycemia. In premature infants, due to higher stress levels than their term counterparts, insufficient glycogen stores may result. Preterm infants are generally considered hypoglycemic when plasma glucose levels are lower than 45mg/dL.\textsuperscript{1} Normal symptoms of hypoglycemia such as seizures, jitteriness, lethargy, apnea, poor feeding are more difficult to detect in a preterm infant. Blood glucose needs to be monitored by routine sampling. One method to treat low blood glucose levels immediately following birth includes intravenous dextrose infusion of 2 mL/kg of dextrose in water solution (200mg/kg), followed by a continuous IV infusion of 6-8 mg/kg/min to maintain a constant supply of glucose for metabolic needs. Rapid infusion of greater than 10% should be avoided due to possible hyperosmolarity that can cause a risk of cerebral hemorrhage.\textsuperscript{1}

*Epidemiology of Bronchopulmonary Dysplasia*

Northway et al. first coined the phrase bronchopulmonary dysplasia (BPD) in 1967 when researching pulmonary disease following respirator therapy.\textsuperscript{6} Need for long-term supplemental oxygen that extends past the original due date of the baby leads to a diagnosis of Bronchopulmonary Dysplasia.\textsuperscript{5} The emphasis on barotrauma or volutrauma concentrating on the pressure or amount of oxygen has changed since the term was presented 45 years ago and has been replaced by a multifactorial etiology.\textsuperscript{7} The key
ingredient is still low gestation associated with underdeveloped lungs. However, the pathobiology has been aggravated by the presence of intrauterine growth restriction, supplemental oxygen exposure, pre- and postnatal pro-inflammatory mechanisms, and nutritional deficits compromising lung maturation and repair. BPD is the most prevalent and one of the most serious long-term sequelae of preterm birth. With an average BPD rate of 23% among infants whose birth weights are below 1500 g, at the current U.S. annual birth rate of approximately 4.1 million, 1.49% of whom are born below 1500 g, greater than 14,000 infants born each year in the United States are destined to develop BPD.

Pathophysiology - Hyperoxia

Due to the fragility of the infant’s underdeveloped lungs, supplemental oxygen, although necessary for survival, can easily irritate and damage lung tissue. Oxygen supplementation above normal amounts has been shown to delay the onset of spontaneous respiratory efforts. Of even more importance may be that brief but excessive oxygen exposure results in reactive oxygen species interacting with immature airway epithelium and interfering with airway-related signaling pathways. One study demonstrated that initially high as compared to low initial supplemental oxygen exposure after delivery may be associated with a greater need for ventilatory support and a higher subsequent incidence of BPD.
Pathophysiology – Ventilation

In early treatment for a high risk preterm infant, healthcare providers seek to quickly establish an optimal functional residual capacity in order to support gas exchange without provoking stretch-induced injurious cascade of lung injury. The rapid aeration of immature lungs can be life saving but must be weighed against lung or airway injury. The use of continuous positive airway pressure-based strategy has been shown in several studies to provide an effective alternative to immediate intubation and surfactant administration for many infants 25-28 weeks gestation range. This may help avoid some long-term airway damage but there are no clinical tests or biomarkers to see which infants would likely succeed and delaying needed surfactant treatment is suboptimal.  

Pathophysiology – Smooth Muscle Contraction

In a developing fetus there is abundant airway smooth muscle that serves in the propagation of lung fluid and resultant lung development. It may also help with stability when the airway has a high compliance due to scarce amounts of cartilaginous and other connective tissue that will develop late in gestation. Neonatal lung injury form excessive supplemental oxygen may change the balance of smooth muscle and airway compliance may change predisposing the infant to later airway hyper-reactivity.

Bronchopulmonary Dysplasia, or BPD, is characterized by inflammation and scarring of the bronchiole tubes of the airway and alveoli of the lungs. This makes gas exchange and respiration difficult. Low birth weight, infection, and congenital heart problems are associated with the development of BPD. It is one of the most common
chronic lung diseases of childhood behind asthma and cystic fibrosis and the most common airway morbidity in surviving preterm infants.\textsuperscript{11}

In summary, the pathophysiology of BPD is considered to be multifactorial. It can be characterized by developmental dysregulation of pro-inflammatory cytokines, cytokine-mediated lung inflammation, pulmonary edema, increased alveolar epithelial and capillary endothelial permeability, volubaro, or oxy-trauma from mechanical ventilation and supplemental oxygen therapy, abnormal expression of local parenchymal, vascular growth factors leading to an arrest in lung development, and pulmonary interstitial thickening resulting in poor gas exchange.\textsuperscript{11}

\textit{Furosemide (Lasix) Use in BPD Patients}

Furosemide (Lasix) is the treatment of choice for fluid overload in infants with bronchopulmonary dysplasia. It is a loop diuretic that improves clinical pulmonary status and function and decreases pulmonary vascular resistance. Daily or alternate-day furosemide therapy may facilitate weaning from positive pressure ventilation (PPV), oxygenation, or both. Adverse effects of long-term therapy are frequent and include hyponatremia, hypokalemia, contraction alkalosis, hypocalcemia, hypercalciuria, renal stones, nephrocalcinosis, and ototoxicity. Careful parenteral and enteral nutritional supplementation is required to maximize the benefits instead of exacerbating the adverse effects.\textsuperscript{11}

BPD initially presents with an exudative phase primarily in the immature preterm neonate still receiving respiratory support around 7 to 10 days of age. During this phase,
pulmonary edema develops due to pro-inflammatory cytokine-induced increased alveolar-capillary membrane permeability. Persistence of a patent ductus arteriosus (PDA) with left-to-right shunting or administration of excessive intravenous fluids during this period also promotes pulmonary overcirculation which contributes to the pulmonary edema. The associated increased lung water content results in decreased lung compliance both in evolving and established BPD. Diuretics are administered to prevent or treat the pulmonary edema and control total body sodium content. Furosemide and other loop diuretics primarily exert their effects by inhibiting the 2Cl⁻,Na⁺, K⁺ cotransporter in the kidney and the lungs, resulting in diuresis and decreased pulmonary edema formation. Treatment with diuretics is associated with decreased pulmonary edema and airway resistance as well as improvements in lung compliance. However, diuretics have potentially significant adverse effects, including fluid and electrolyte imbalance, osteopenia, nephrocalcinosis, hearing impairment, and growth failure. Phosphorous, magnesium, and calcium excretion also are increased by complex and not completely understood mechanisms.¹¹

Along with edema and oxygen exchange issues, caregivers have to consider the nutritional needs of an underdeveloped and small infant with BPD.

*Parenteral Nutrition*

Sufficient nutrition is difficult to achieve in preterm infants. A sufficient amount of protein and calories seems to be necessary for organ growth. Poor nutrition could impair the developing lung because it can modulate lung structure.¹² When in the
neonatal intensive care unit (NICU), daily and weekly monitoring of temperature, blood glucose, fluids, and electrolytes are carefully evaluated. Most fetal nutrition is deposited in the last three months of pregnancy. An ELBW or VLBW infant may only have 1% of its total body weight in fat stores whereas a term infant may have 16% or substantially more contributed to nutrition stores. To further complicate this problem, organized gut motility doesn’t begin until 32 to 34 weeks of gestation. Therefore, a very premature infant will require parenteral nutrition immediately after birth.

Concern for adequate nutrition to allow proper growth in a preterm infant with BPD is a continual challenge. Infants are weighed daily and other measurements such as body length and head circumference are monitored weekly. Some lag in growth is expected due to the comorbidities associated with ELBW and BPD but adequate caloric intake is extremely important for a developing infant.

Evidence that early enteral feeding may cause a condition called necrotizing enterocolitis (NEC) often lead healthcare providers to defer enteral feedings until the gastrointestinal tract has more time to mature. NEC is the necrosis of a premature infants bowel and is inversely proportional to the gestational age of the infant. It is also the most common intestinal emergency in the preterm infant occurring in 1-3 infants per 1000 births. This condition is life threatening and perforation of the bowel requires emergency surgery to resect the necrotized portion of the bowel. NEC is the cause for approximately 2,600 neonatal deaths annually with a mortality rate of 10-50%.

Therefore, parenteral nutrition becomes the primary and sometimes sole source of nutrition in an infant with very low and extremely low birth weight. Using a specialized
mixture of amino acids, dextrose, minerals, and electrolytes known as total parenteral nutrition attains the optimal parenteral nutrition. A 20% lipid emulsion is run separately to complete the required nutrition often accompanies the TPN mixture. Because these infants lose at least 1.2 g/kg/day of endogenous protein, at least that amount of amino acids is required plus 30kcal/kg/day to maintain protein homeostasis. Prolonged use of parenteral nutrition may result in cholestasis and elevated triglyceride levels. Regular lab tests are performed to evaluate liver function and triglyceride levels.\textsuperscript{1}

\textit{Micronutrition}

Along with the caloric requirements necessary for proper organ development, vitamins and minerals are essential for undisturbed growth. Nutrients such as inositol, fatty acids, vitamin E, and vitamin A have been suggested to be scavenger antioxidants and helpful stimulants in re-epithelialization of lung tissue in the prevention and recovery from Bronchopulmonary Dysplasia.\textsuperscript{14} Minerals also play a crucial role in the therapy of low birth weight infants in varying amounts. Calcium and phosphorous are the most widely studied minerals in the literature due to the immediacy of their effect to prevent acute metabolic disorders such as hypocalcemia, as well as their late-onset manifestations such as metabolic bone disease. However, trace minerals such as zinc, magnesium, iron, selenium, copper, and others also play an important role in development.\textsuperscript{15}

Mineralization levels increase dramatically between 24 and 37 weeks of gestation. This increase accounts for 80\% of mineral accumulation happening in the third trimester. Therefore, infants born before 37 weeks will be deprived of the intrauterine supply of
calcium and other minerals needed for skeletal, dental, other organ system growth. The goal then of the early postnatal period in the preterm infant is to provide not only adequate carbohydrates, proteins, and fats, but also to provide appropriate levels of vitamins, minerals, and trace elements to avoid morbidities associated with undernourishment.

Selenium has been shown to be an important constituent part of selenoenzymes, glutathione peroxidase, that act as antioxidants to protect cell membranes. These enzymes prevent the formation of free radicals protecting the body from oxidative insult. The control mechanism of mother-to-fetus transfer of selenium is poorly understood and no data exist on the fetal concentration of selenium. Mineralization is estimated at 1mcg/kg/day. Selenium is stored in the liver between the 20th and 40th week of gestation. It is estimated that the activity of selenium required glutathione peroxidase corresponds to one third of that which is observed in full-term newborns.

Zinc is also an important microelement for growth, cell differentiation, and metabolism of proteins, carbohydrates, and lipids. Clinical signs of zinc deficiency may include weight loss, failure to thrive, periorificial dermatitis, glossitis, and enhanced susceptibility to infections. Similar to other micronutrients, zinc accumulation occurs in the third trimester leaving preterm infants deprived of necessary zinc stores. Immature gastrointestinal tracts of preterm infants also excrete zinc leaving an even more negative balance of zinc. Bioavailability of zinc can be reduced by interference from other micronutrients such as iron. Calculations of zinc requirements indicate a requirement of 600 mcg/day for the formation of new tissue in preterm infants born between 24 and 28
weeks. The release of hepatic zinc by a newborn weighing 1,000 grams is on average 150 mcg/day.\textsuperscript{15}

Copper is an essential trace element and important enzyme cofactor. Deficiency found in preterm infants can be identified as anemia, neutropenia, growth failure, hair depigmentation, apnea, edema, and hypothermia as well as bone abnormalities.\textsuperscript{16} Copper testing requires large blood volumes for small infants (4mL), which discourages routine monitoring.\textsuperscript{16} The World Health Organization has recommended a minimum intake of 60 mcg/kg/day for infants whereas the new recommended daily allowance for copper is 200 mcg/day.\textsuperscript{17}

Iron deficiency results in anemia that can be further exacerbated by frequent phlebotomy and inadequate intake. Iron deficiency anemia is actually a late finding. At this point, brain iron stores may already be severely depleted and the effects on development may be irreversible. (Shah and Shah, 2009)

It has been shown that metabolic bone disease occurs in approximately 50% of extremely low birth weight infants and 30% of infants with very low birth weight due to inappropriate levels of calcium and phosphorus. Also, the solubility of calcium in the presence of phosphorus is a limiting factor in parenteral nutrition. In addition several factors such as the source of calcium, concentration, solution pH, glucose concentration, and temperature of the solution can affect solubility of calcium.\textsuperscript{15} One evaluation concludes that 60 to 70% of intrauterine mineralization could be obtained from usual parenteral solutions.\textsuperscript{15} Even if the actual percentages are higher, proper calcification of bone and enamel is a concern.
Early enteral feedings of breast milk supplemented with calcium and phosphorus increase the amount of calcium retained by the infant but this is sometimes not possible for preterm infants. Also, it is unclear the degree of effect that supplemented breast milk has on the calcification of hard tissue.\textsuperscript{15}

Short-term effects of low calcium and phosphorus results in low bone mineral density (BMD) but the long-term effects are less clear. Some studies have suggested that the disparity of BMD disappears in early childhood.\textsuperscript{15} Direct measurements of blood calcium levels, as an indicator of mineral loss is not useful since levels remain fairly constant even in extreme calcium deficiency. The calcium is removed from calcified tissues to maintain serum homeostasis even in deficiency.\textsuperscript{18} Calcification of enamel in teeth however occurs during a defined period and is limited after that because ameloblasts, the cells that deposit and calcify enamel, are no longer present after the tooth is formed. So, any hypomineralization or insults to enamel formation would be permanently recorded.\textsuperscript{19,20}

*Enamel Hypoplasia/Hypomineralization and Other Dental Disturbances*

The effects of therapy for preterm infants have shown to include enamel opacity and enamel hypoplasia, crown dilaceration, palatal distortions resulting in crossbite, and delays in primary and permanent dentition eruption.\textsuperscript{21} Premature infants who were subsequently intubated have been shown to have a higher long-term prevalence of oral defects than their full-term counterparts in both the primary and mixed dentitions. Enamel defects, high vaulted palate, grooved palate, cross-bite, speech intelligibility, and
speech nasality have been shown to be significantly more prevalent in children from 3-5 years old who were preterm and has a history of intubation.\textsuperscript{22}

That higher prevalence has been shown to persist in children of mixed dentition from 7-10 years old. The exception to this is when considering enamel defects of permanent maxillary incisors.\textsuperscript{22} The presence of enamel defects in the primary dentition, as well as absence in the mixed dentition raises a question of causation. Also, trauma from intubation as the causative factor has been evidenced when the location of enamel defects is in the incisal third of the maxillary anterior primary incisors.\textsuperscript{22}

The current study is focused on enamel hypoplasia and enamel quality of mineralization and correlations in current practice BPD therapy that may suggest causation. The prevalence of enamel defects ranges from 43\% to 96\% of VLBW infants.\textsuperscript{23} It has also been associated with serious illness during the neonatal period individually such as respiratory distress, apnea, hypoglycemia, intracranial hemorrhage, cardiac defects, and infections.\textsuperscript{18,20} Using light and scanning electron microscopy, it has also been shown that thickness of enamel in children with a history of prematurity is significantly thinner than full-term enamel.\textsuperscript{24} It is not difficult finding associations in medical conditions found in preterm infants and enamel hypoplasia when compared with healthy full-term control children. However, correlations in therapy for BPD patients and the presence or lack thereof of enamel hypoplasia may lead to inferences about causation.

One study looked at cortical area of long bones of VLBW infants and compared those infants found to have enamel hypoplasia to those who did not. It was found that those with lower mean cortical bone area suggesting hypomineralization were also
significantly more likely to have enamel hypoplasia. Ameloblasts are sensitive to even short periods of calcium change.

The clinical significance of enamel hypoplasia goes beyond the unfavorable esthetic outcome as well. Enamel hypoplasia is linked to increased plaque accumulation, dental caries, and in more severe cases, with space loss and malocclusion. In a longitudinal study by Lai et al there was a significant association with enamel defects and dental caries in the VLBW group. The most dental caries were observed in children who had both hypoplasia and opacities from hypomineralization.

Since mineralization of enamel in primary teeth begins in the fourth month of gestation and continues of throughout the third trimester, changes in the micronutrients and trace minerals in a preterm infants being treated for BPD may affect enamel maturation. Enamel of permanent teeth that begin calcification around the time of normal gestation such as the first permanent molar have also been show to have higher prevalence of hypoplasia in children prematurely. Associations found in therapies such as Furosemide use and total parenteral nutrition and the presence of enamel hypoplasia/hypomineralization may give insight into the effects these treatments have on already low mineral stores of preterm infants with BPD.
Materials and Methods

This IRB approved, case cohort study was done at two different clinics at Nationwide Children’s Hospital. Subjects with a history of BPD were examined at the Bronchopulmonary Dysplasia clinic as well as at a dental screening booth at a Small Baby Reunion honoring children who were born preterm with low birth weights. Healthy control subjects were examined at the baby clinic within the dental clinic. A convenience sample of children ranging in age from 12 to 49 months was taken. All legal guardians approached agreed to participate in this study. Medical and dental histories, history of intubation, oral hygiene habits, and feeding practices were obtained. History of prematurity, BPD, and/or intubation disqualified participation in the control group. Demographic information including gender and race of the patients were noted. Hospital EPIC charting was then used for BPD subjects to determine days on total parenteral nutrition, doses of Lasix, history of necrotizing enterocolitis, and GERD.

One practitioner identified all BPD subjects. The same practitioner examined most of the control group. Another practitioner at NCH and a fourth year dental student who were calibrated to identify hypoplasia in the same manner examined eight subjects in the control group. The teeth were dried with gauze and a dental explorer was used to evaluate surface of enamel. Presence or absence of enamel hypoplasia and/or hypomineralization was then determined using the scale in Table 1 designed by the
European Academy of Pediatric Dentistry to help with diagnosis on molar incisor hypoplasia/hypomineralization. (Alves dos Santos, 2011) Enamel hypoplasia was diagnosed if there was a break in continuity of the enamel surface such as pitting, ridging, or other disturbances of enamel contour. Enamel hypomineralization was diagnosed as opacities and changes in translucency of enamel with white or yellow/brown discoloration without breaks in the continuity of the enamel surface.18 All surfaces of erupted teeth were examined and areas with frank cavitation were noted as carious instead of hypoplasia. Location of the hypomineralized/hypoplastic areas was charted using an orthodontic decalcification index to simplify recording. (Robertson, 2011) Parents were also provided oral hygiene and diet recommendations, correct teeth brushing techniques, and other anticipatory guidance guidelines to prevent dental decay.

| Code | Criteria |

Table 1: Grading Using Molar-Incisor Hypoplasia Scale
Figure 1: Orthodontic Decalcification Index
Results

Demographics

Data were collected over a 9 month period from June 2012 to March 2013. Data were collected from 67 children (32 BPD and 35 controls). The mean age of all subjects was 28.2 months ± 10.6 months. The entire cohort was comprised of 43 males and 24 females. There was no significant gender difference between the two groups. The mean age of the BPD group was 25.9 months ± 11.2 months and control age was 30.1 ± 9.5 months. The BPD group age at time of exam was significantly younger (p=.048). The BPD group also had a significantly younger gestational age (25 weeks) compared to the control group (39.5 weeks) (P<.0001). In the BPD cohort, 17 subjects were Caucasian/white, 12 were African American, 0 were Asian, 2 were Hispanic/Latino, and 1 was Middle Eastern. In the control subject cohort, 16 subjects were Caucasian/white, 11 were African American, 0 were Asian, 7 were Hispanic/Latino, and 1 was Middle Eastern. Demographic data for the subjects are summarized in Table 2. When compared to the control group, The BPD group had significantly higher incidences necrotizing enterocolitis (P=.021). Sixty-five percent of children in the BPD group were diagnosed with gastro-esophageal reflux, compared to none in the control group.
### Table 2: Demographic Data Results

<table>
<thead>
<tr>
<th>Group</th>
<th># Subjects</th>
<th>#Male/#Female</th>
<th>Average Gestational Age</th>
<th>Average Age at Exam</th>
<th>Ethnicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>BPD</td>
<td>32</td>
<td>23/10</td>
<td>25 weeks</td>
<td>25.9 ±11.2 months</td>
<td>17 White, 12 AA, 2 Latino, 1 MiddleEast</td>
</tr>
<tr>
<td>Control</td>
<td>35</td>
<td>20/14</td>
<td>39.5 weeks</td>
<td>30.1 ±9.5 months</td>
<td>16 White, 11 AA, 7 Latino, 1 MiddleEast</td>
</tr>
<tr>
<td>Total</td>
<td>67</td>
<td>43/24</td>
<td>P&lt;0.0001</td>
<td>P = 0.048</td>
<td>33 White, 23 AA 9 Latino, 2 MiddleEast</td>
</tr>
</tbody>
</table>

**Variables of Early Childhood Nutrition**

The control group had no incidences of IV/IM Lasix, or total parenteral nutrition (TPN). The mean BPD child had 8.8 doses of IV or IM Lasix and in addition, received 8.7± 8.7 doses of Lasix orally or through a gastrostomy tube. Both of these variables were statistically significant (P<.0001). Overall, 53.7% of subjects were reported to have been breastfed (alone or in combination with formula). Sixty-five percent of the BPD group had breastfeeding experience, compared to 43% of the controls. (P=.087). Caregivers were asked to elaborate on the duration of breastfeeding. Children in the BPD group were nursed a mean of 2.7±4.3 months compared to the control children who were nursed a mean of 6.3±7.0 months (P=.010). The BPD group had significantly higher orders for TPN compared to the controls (P<.0001). The BPD group had a mean of 42.5± 40.6 orders for total parenteral nutrition. This compared to less than 1 (0.2) orders for the control group. Children in the BPD group also received significantly higher orders for therapeutic sucrose (p<.0001), with a mean of 3±2.2 doses.
<table>
<thead>
<tr>
<th>Group</th>
<th>Lasix Dose</th>
<th>Reported Breastfeeding</th>
<th>Duration of Breastfeeding</th>
<th>Parenteral Nutrition</th>
<th>Therapeutic Sucrose</th>
</tr>
</thead>
<tbody>
<tr>
<td>BPD</td>
<td>8.8 IM/IV</td>
<td>65%</td>
<td>2.7±4.3 months</td>
<td>42.5± 40.6 orders</td>
<td>3±2.2 doses</td>
</tr>
<tr>
<td></td>
<td>8.7 oral/G-tube</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0 IM/IV</td>
<td>43%</td>
<td>6.3±7.0 months</td>
<td>0 orders</td>
<td>0 orders</td>
</tr>
<tr>
<td></td>
<td>0 Oral/G-tube</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p-Values</td>
<td>p&lt;0.0001</td>
<td>p=0.087</td>
<td>p=0.010</td>
<td>p&lt;0.0001</td>
<td>p&lt;0.0001</td>
</tr>
</tbody>
</table>

Table 3: Nutrition Data Results

_Dental Variables_

Children in the BPD group had significantly fewer teeth present (14.7± 5.2) compared to controls (17.4±4) (P=.006). Children in the BPD group had significantly higher incidences of hypomineralization (P=.0007), both the white creamy variant (P=.015) and the yellow brown variant (P=.005) were more notable in the BPD group. There was no significant difference in the overall incidence of post-eruptive breakdown, as defined by Alves dos Santos in the European Academy of Pediatric Dentistry(P=0.224). The BPD group also had a significantly higher incidence of enamel hypoplasia (P=.011), and it also affected more teeth in the BPD group (P=.018). There was no difference in the overall caries experience between the two groups, either in cumulative incidence (P=.754) or number of affected surfaces (P=0.457). In both groups, less than half had seen a dentist prior to this examination (P=1). There was no significant difference in the mean number of times children had their teeth brushed (P=0.142). Subjects who had received Lasix were divided into groups of under 8 doses and 8 doses and above. There was no statistical significance in number of teeth (p=.071), hypomineralization((y/n) = p=0.756), or hypoplasia ((y/n)= p=0.423). There was also no difference in the number of
hypoplastic/hypomineralized teeth (p=0.192). Number of carious teeth was also not significant (p=0.064).

<table>
<thead>
<tr>
<th>Group</th>
<th>Teeth Present</th>
<th>Enamel Hypomin</th>
<th>White-Creamy Variant</th>
<th>Yellow-Brown Variant</th>
<th>Enamel Hypoplasia</th>
</tr>
</thead>
<tbody>
<tr>
<td>BPD</td>
<td>14.7± 5.2</td>
<td>44%</td>
<td>53%</td>
<td>39%</td>
<td>33%</td>
</tr>
<tr>
<td>Control</td>
<td>17.4±4</td>
<td>5%</td>
<td>9%</td>
<td>3%</td>
<td>3%</td>
</tr>
<tr>
<td>p-Values</td>
<td>p=0.006</td>
<td>p=0.0007</td>
<td>p=0.015</td>
<td>p=0.005</td>
<td>p=0.011</td>
</tr>
</tbody>
</table>

Table 4: Dental Results
Discussion

The gross parameters of micro-nutrition and the diuretic Furosemide used in BPD therapy were not directly associated with dental disturbances in children with BPD.

![Figure 2: Correlation TPN vs Hypomineralized Enamel](image)

$R^2 = 0.2098$
Figure 3: Caries vs Number of Therapeutic Sucrose Doses


