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The Role of Vitamin D Deficiency in Early Childhood Caries

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The Role of Vitamin D Deficiency in Early Childhood Caries

A Thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Dentistry at Virginia Commonwealth University.

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Abstract

THE ROLE OF VITAMIN D DEFICIENCY IN EARLY CHILDHOOD CARIES

By Tiffany L. Williams, DDS

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Dentistry at Virginia Commonwealth University.

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Purpose: This is a pilot study to determine if there is a relationship between vitamin D deficiency and the development of early childhood caries (ECC). **Methods:** Serum 25(OH) vitamin D, parathyroid hormone and calcium were measured from children with ECC, who presented for dental rehabilitation under general anesthesia. Samples were obtained from caries free controls examined during a well-child medical check. Parents from both sample sizes filled out a questionnaire consisting of 22 questions regarding the child and parent's medical and dental history as well as socioeconomic background. **Results:** ECC patients were found to have deficient serum levels of 25(OH) vitamin D and calcium compared to caries free controls. **Conclusions:** This study observed a difference in 25(OH) vitamin D levels between the two groups. Due to limited sample size and confounding variables contributing to ECC, a definitive relationship between vitamin D deficiency and ECC cannot be made at this time.

Introduction

Early childhood caries (ECC) is defined as the presence of 1 or more decayed (noncavitated or cavitated lesions), missing (due to caries), or filled tooth surfaces in any primary tooth in a child 71 months of age or younger.[22] In children younger than 3 years of age, any sign of smooth-surface caries is indicative of severe early childhood caries (S-ECC). ECC has become the most common chronic childhood disease in the United States, five times more prevalent than asthma[35]. Due to the rising amount of children presenting with early childhood caries (ECC) [14], preventing the initiation and progression of dental decay is important to the oral health and overall health of children. Many causative factors exist to play a role in the development of dental decay, including bacteria, diet, oral hygiene, medical conditions and lack of important vitamins and minerals, such as vitamin D.

At least 1 billion people worldwide have vitamin D deficiency or insufficient levels of vitamin D[7]. Vitamin D deficiency is defined as a 25-hydroxyvitamin D (25(OH) D) level of less than 20 ng per milliliter[8]. There are many causes of vitamin D deficiency, including heritable disorders, acquired disorders and reduced synthesis of vitamin D absorbed through the skin [7, 11]. Vitamin D deficiency can contribute to a number of conditions including vitamin D resistant rickets, osteoporosis, dental enamel hypoplasia and dental caries.

Vitamin D is a steroid hormone essential for bone growth and remodeling, as well as tooth development, especially the early stages of morphogenesis, differentiation and enamel and

pulp development[2]. The major physiologic function of vitamin D is to maintain serum calcium and phosphorus levels; without vitamin D, intestinal absorption of dietary calcium and phosphorus is significantly decreased.[7] When serum calcium levels are decreased, parathyroid hormone (PTH) levels increase, in turn, increasing tubular resorption of calcium and enhancing the action of osteoclasts to mobilize calcium stores from the bone. In addition, PTH stimulates the kidneys to convert 25(OH) D to its active form of vitamin D, 1, 25-dihydroxyvitamin D.[27, 9]

Vitamin D has been shown to stimulate increased saliva flow in animals[2]. It is hypothesized that salivary flow can provide calcium ions, and in the case of fluoridated water supplies, fluoride ions for remineralization of incipient caries and provide activity against oral bacteria.[11] The effects of significantly higher saliva flow was evident in a study in children showing the lower salivary flow with DMFT (decayed, missing, filled teeth) >5 compared to higher with zero DMFT.[4]

Humans acquire vitamin D from multiple sources. The liver directly synthesizes vitamin D as we obtain it from our diet and as sunlight hits the skin. Dietary sources of vitamin D include oily fish, like salmon or canned tuna, shiitake mushrooms, egg yolk, foods fortified with vitamin D, and dietary supplements.[7] The current recommendation from the American Academy of Pediatrics (AAP) and the Institute of Medicine is 400 International Units (IU) of vitamin D per day during the first year of life and 600 IU after the first year of life.[28] Human milk does not contain an adequate amount of vitamin D to act as the main source of supplement for infants who are breastfed since it only provides 25 IU/L or less.[28] Children who are breastfed are at increased risk for vitamin D deficiency if they are not receiving adequate sunlight exposure or vitamin D supplements. Furthermore, children who receive inadequate vitamin D through sun exposure or diet may be at risk for early childhood caries[7].

The first research to find a correlation between vitamin D and dental caries was completed in the late 1920's when investigators proved that dietary factors that control the structure of teeth in puppies also influence the initiation and spread of caries in children [5]. Mellanby and colleagues discovered that through controlled diet of hospitalized children, those who were fed diets fortified with vitamin D (eggs, cod liver oil, milk, cheeses), had a decrease in carious lesions and arrested carious lesions after dmft scores were measured. This study found that vitamin D containing foods stimulated calcification of children's teeth and suppressed the active carious process [5.]

Hujoel et.al hoped to further investigate the link between vitamin D and tooth decay. The findings from this systematic review and meta-analysis reaffirm the importance of Vitamin D for dental health- children who are vitamin D deficient have poor delayed tooth eruption and are prone to dental caries[8]. Hujoel states:

"In summary, this systematic review of controlled clinical trials suggests that vitamin D exposures in early life may play a role in caries prevention. This promising evidence base may be relevant to current challenges in improving health, as vitamin D levels in the US population are decreasing and dental caries among US children is increasing."

Schroth and colleagues conducted a pilot study in which 38 participants (19 controls and 19 patients with severe early childhood caries) were assessed for adequate levels of vitamin D and parathyroid hormone. His study reported that children with severe ECC have lower vitamin D levels compared to cavity free controls[9].

The goal of this study is to investigate the prevalence and severity of early childhood caries in children with vitamin D deficiency. We hypothesize that children with early childhood caries will have lower levels of 25(OH) D.

Research Design and Methods

This project was approved under expedited status from the Virginia Commonwealth University Institutional Review Board (VCU IRB #: HM15486). This research involves children.

This is a case-control pilot study to determine the relationship of Vitamin D deficiency to the prevalence and severity of early child caries. Data was collected at VCU Health Systems operating room and Children's Pavilion from November 21, 2013 to February 26, 2014.The following inclusion and exclusion criteria were followed:

Inclusion Criteria

- Cases: Patients diagnosed with early childhood caries who are treatment planned to receive full mouth dental rehabilitation under general anesthesia
- Age 71 months or younger
- Patients with an ASA (American Society of Anesthesiologists) Classification of 1 (healthy person) or 2 (mild systemic disease)
- Controls: patients who present with no frank cavitated lesions upon visual exam who are:
    - patients of record at VCU Children's Pavilion scheduled for an annual exam with blood work
    - patients scheduled to undergo an otolaryngology procedure under general anesthesia

Exclusion Criteria

- Patients with significant metabolic disorders and/or complex medical issues

- Patients with an ASA Classification of 3 or greater
- Controls: patients who present with frank cavitated lesions upon visual exam

Patient selection and examination

There were two groups of patients recruited into this study, cases and controls.

Case patients must present to VCU Pediatric Dental Clinic for a new patient dental exam, and meet the criteria of a diagnosis of early childhood caries (ECC). At this first dental visit, patients with ECC who are treatment planned for full mouth dental rehabilitation (FMDR) under general anesthesia by a pediatric dental resident were approached for participation in the study.

Patients from the VCU Pediatric Dental Clinic who presented with early childhood caries and who were treatment planned to receive dental care under general anesthesia were recruited to participate in the study. Children and their caregivers were approached and the study was explained in verbal and written form. Consent was obtained to allow blood samples to be drawn while the patient is under general anesthesia for their dental treatment. All pediatric dental residents were trained to discuss the study in detail with participants and obtain adequate consent. A calibrated dental examination of teeth was completed in which dmft (decayed, missing, filled surfaces/teeth) scores were charted. Patients underwent dental rehabilitation under general anesthesia regardless of their participation in the study.

In order to assess biological and non-biological caries risk factors outside of Vitamin D deficiency, the consenting caregivers completed a demographic and behavioral questionnaire related to dental caries risk assessment.

Control patients were identified from those who presented to VCU Children's Pavilion for yearly exams and blood work, as patients of N. Romesh Wijesooriya, MD, were considered

as caries-free controls. Consent was obtained from the parent or guardian after explaining the study in detail. When consent was obtained, a short questionnaire was administered to the parent. Patients had a yearly pediatric medicine exam and blood drawn regardless of their participation in the study.

Blood samples

The operating room staff obtained blood samples while the patient was under general anesthesia prior to the initiation of dental treatment. An intravenous (IV) line was placed while the child is in the operating room for clinical purposes. No additional needle sticks were incurred, as blood was drawn from a clinically necessary IV line. Serum was obtained as a maximum fill of a 3.0 mL tube of a violet topped BD Vacutainer tube containing 5.4mg of EDTA and gold topped 4.0mL BD Vacutainer tube. Patients were assigned a Research Medical Record Number in the Cerner Hospital database so that blood samples are correctly analyzed and tabulated when they left the operating room.

Blood work obtained from the caries free controls during their annual exams was also assigned a research related identification number in the same manner.

Vitamin D analysis

All blood samples were analyzed for 25-OH Vitamin D levels in VCUHS Clinical Pathology Laboratory by Dr. Lorin Bachmann (Co-Director of Clinical Chemistry) and staff. A liquid chromatography tandem mass spectroscopy was used to analyze total 25-OH Vitamin D levels. Total 25-OH Vitamin D was calculated as the sum total of 25-OH Vitamin D3 and 25-OH Vitamin D2. The method is traceable to the National Institutes of Technology SRM 972a serum reference materials for 25-OH Vitamin D. Lab results were entered into a database and charted

for data analysis. Parathyroid hormone (PTH) and calcium levels were analyzed with the same blood sample in addition to 25(OH) D levels. PTH is a more sensitive surrogate for mild Vitamin D deficiency and is typically analyzed in conjunction with Vitamin D. Analysis and reported data was based on the following values: <10 ng/mL severe hypovitaminosis D, 10.0-19.9 ng/mL moderate hypovitaminosis D, 20.0-29.9 ng/mL mild hypovitaminosis D, 30.0-100.0 ng/mL optimal, >100 ng/mL potential for toxicity may exist[37].

Blood work drawn was analyzed and any discrepancy in findings, such as Vitamin D deficiency, was reported to the patient so that they were referred for treatment by their pediatrician.

Analytical Plan

The following patient characteristics will be described separately in the ECC and healthy group: Patient's demographics, dmft scores, Vitamin D levels and PTH levels.

To test Aim 1 (ECC vs healthy on vitD), a two-group t-test will be used. To test Aim 2 (ECC vs healthy on PTH), a two-group t-test will be used. Aim 3 is secondary aim to test for vitD and PTH differences. This aim will be tested using ANCOVA (testing for an ECC vs healthy difference, adjusting for the covariates). All analyses will be done using SAS software (SAS Institute Inc., Cary NC) at alpha =.05.

Sample size calculation: Using the mean 25(OH) D values in Schroth, we expected S-ECC mean of 52.9nmol/L and a healthy mean of 64.4. Assuming equal sample size in each group, and testing for a difference using a t-test at alpha=0.05, in order to achieve an 80% power to detect a difference the following sample sizes per group are required: If the common SD within each group is 15.1, then n=29 per group are required. If the common SD within each

group is 21.3, then n=55 per group are required. If the common SD within each group is 18.2 then n=41 per group are required.

Results

The results of the study will be shown in two sections, a description of the two groups of participants and the analyses comparing the cases and controls.

Description of participants

Thus far, two healthy controls and 10 ECC patients have been entered into the study. Table 1 shows the demographic characteristics of the participants. The average age of the controls was 15.4 months (SD = 4.6) and the ECC patients averaged 42.1 months old (SD = 12.8). Ethnicities of the participants included… Of the ECC group, 1 parent reported only an elementary/middle school education, 6 reported a high school education, 1 reported trade school, 1 reported college level education and only 1 reported graduate school beyond college. Among the control group, 1 parent reported a college level education and 1 reported graduate school beyond college. Household income level was inquired. Three of the ECC group reports incomes less than 5,000 per year, 1 reported 10,000-19,999 per year, 1 reported 20,000-29,999 per year, 1 reported 50,000-79,999 per year and only 1 reported making $100,000 or more. Both parents of the control group reported making $100,000 or more per year.

The medical history of the two groups of patients is shown in Table 2. Of the ECC patients, two reported a respiratory disorder, such as asthma. One ECC patient reported ADD/ADHD and 2 ECC patients reported premature birth or illness as a newborn. One ECC patient marked ‘other’ as a medical problem, but did not include a descriptor. Four ECC patients and 1 control patient reported daily sun exposure, where 3 ECC patients reported weekly sun

exposure. The healthy control patients did not report significant medical history outside of taking daily vitamins.

The dental history of the two groups of patients as well as the eating habits of the children is shown in Table 3 and 4 respectively. Tables 3 and 4 include factors that in addition to Vitamin D deficiency may play a role in a child's caries risk. When dental history and eating habits were questioned, it was rated as 'yes or no' or as a frequency, 'daily, weekly or monthly'.

Three of the ECC group marked *yes* to fluoridated water, 2 marked *no* and 2 *did not know*. One control patient marked *yes* to water fluoridation, while the other control patient did not respond to this question. One ECC patient marked *yes* to fluoride supplementation outside of dental visits, 8 marked *no* and 1 *did not know*. One of the control patients answered *yes* to fluoride supplementation and 1 marked *no*. Five of the ECC group received fluoride treatments regularly, 4 do not and 1 patient *did not know*. Both of the control patients do not receive regular fluoride treatment.

When brushing frequency was reported, 9 of the ECC patients report brushing daily and 1 weekly. One of the control patients report brushing daily and the other marked *never*. Four of the ECC group reported flossing daily while 6 reported never flossing. Both of the control patients reported never flossing.

Eight of the ECC group parents reported that their child visits a dentist twice a year, while two reported yearly visits. Both of the control patients reported their children never visiting a dentist. Two of the ECC group reported difficulty getting their children to the dentist, while 8 reported no difficulty. The two control groups reported no difficulty getting the child seen at the dentist, as they had never visited the dentist before.

Parental dental history and hygiene was also reported. Seven parents of the ECC group reported *yes* to having active caries, and 3 of the parents reported *no*. Both parents of the control group answered *no* to having dental caries. Parents of both the ECC and control groups reported having all of their natural teeth in addition to brushing daily. Eight of the ECC group listed Medicaid as their primary insurance, while 2 of that group marked *no* to Medicaid. None of the control group indicated Medicaid being their primary insurance. Six parents of the ECC group reported having dental insurance and 4 marked *no*. Both parents of the control group marked *yes* to having dental insurance. In addition, 4 of the ECC group reported *yes* to participating in a public assistance program such as WIC or Healthy Start. Six of the ECC group marked *no* to this question. None of the control group indicated receiving public assistance.

Children's eating habits were reported in relation to bottle or sippy cup usage, frequency of snacks, sugary drinks and tap water consumption. Six of the ECC group reported bottle/sippy cup usage, while 4 reported *no*. One control patient reported *yes* and 1 *no* for bottle/sippy cup usage. Six out of 10 of the ECC group reported never taking a bottle to bed and 4/10 reported taking a bottle to bed daily. One control reported never taking a bottle to bed and 1 reported taking a bottle to bed weekly. Two ECC patients reported never having snacks before bedtime, 1 reported monthly snacks before bedtime, 2 reported weekly snacks before bedtime and 4 reported daily snacks before bedtime. Of the control group, 1 reported never having snacks before bedtime and 1 reported daily snacks before bedtime.

Increased frequency of snacks throughout the day was noted in the ECC group with 7/10 reporting snacking one or two times a day, 1 snacking weekly and 2 snacking three or more times a day. One control patient reported never snacking throughout the day and 1 snacking one or two times a day. Fifty percent of the ECC group reported having sugary drinks one or two

times a day; 1 reported weekly sugary drinks and 4 reported sugary drinks three or more times a day. One of the control group reported having sugary drinks monthly and 1 reported never having sugary drinks. Tap water was consumed daily by 5/10 of the ECC group, weekly by 1 and never by 4. One of the control group reported daily consumption of tap water and 1 reported weekly consumption of tap water.

Comparison of the groups

In this section the two groups are compared on their Vitamin D levels, PTH, and Calcium. Table 5 shows the Vitamin D levels. The control patients had an average of 39.9 ng/mL of vitamin D and the ECC patients averaged 25.0 ng/mL, 14.9 units lower; although this difference was not statistically significant (t-test $P > 0.1$). In the lower portion of the table, the contingency table shows the number of patients who were either low or normal on vitamin D. The chi-square test indicated a significant difference (P = 0.0217).

Table 6 shows the PTH levels. The mean level for control patients was 43.70 pg/mL. The mean level of PTH for ECC patients was 54.68 pg/mL. Both of the control patients had optimal PTH levels, while 6 of the ECC patients had normal levels. One ECC patient had sub-optimal PTH levels and 3 ECC patients had elevated PTH levels.

Table 7 shows the calcium levels. The ECC patients had significantly lower calcium than did the controls (P = 0.0270). The mean calcium levels of the control group were 10.20 mg/dL, while the mean calcium levels of the ECC group was 9.54 mg/dL. Twelve children had normal calcium levels, and 1 child, in the ECC group, had a low serum calcium value.

The correlations between the measurements are shown in Table 8 and Figure 1. Relevant positive correlations were made between Ca^{2+} and 25-OH Vitamin D levels and Ca^{2+} and PTH levels. Negative, inverse correlations were found between vitamin D and PTH levels.

Discussion

In addition to its immunogenic, bone growth and remodeling properties, the importance of Vitamin D has been implicated in a number of conditions including vitamin D resistant rickets, osteoporosis and a number of dental conditions.[11] Vitamin D is synthesized when the skin is exposed to ultraviolet B radiation, where 7-dehydrocholesterol in the skin is converted to vitamin D_3[7]. Dietary sources of vitamin D are in vitamin D_2 or D_3 form. The major circulating form of Vitamin D is 25-hydroxyvitamin D, or 25(OH)D. Vitamin D_2 and D_3 are converted in the liver to this circulating form, which is the form analyzed in serum to determine vitamin D status.[11,7]

Early childhood caries is a multifactorial disease that affects the teeth of children ages 0-71 months[35]. A patient's age, behavior and/or medical needs may necessitate that dental rehabilitation to treat rampant dental caries be completed under sedation or general anesthesia[36]. The rising incidence of caries in children ages 2-5 has caused pediatric dentists to consider models of prevention in addition to oral hygiene instruction and dietary counseling.[14] Our aim was to determine if insufficient levels of vitamin D, an important secosteroid hormone necessary for the absorption of calcium, contributed to the incidence of early childhood caries (ECC).

Data from our study suggests that there is a statistically significant difference in the 25(OH)D levels of caries free children and children with early childhood caries ($p=0.0217$). In addition to determining whether Vitamin D played a major role in the development of ECC, associated variables were considered. Our data also supports evidence that socioeconomic and dietary factors play a role in the spread and prevalence of dental caries. We found that children from an African American background experience caries more than Caucasians. With our sample size, a wide range of ethnic backgrounds were not included, but a NHANES study confirmed

that African American and Mexican-American children were twice as likely to experience caries as non-Hispanic counterparts[16].

In addition to the difference in the caries experience among ethnic groups, there were differences in household income level and parental education level of the ECC group compared to the controls. Our findings are consistent with the 1988-1994 NHANES study that found a higher caries experience and higher levels of untreated caries in children from lower-income households[16]. Low parental education level has also been associated with increased caries experience in children[22, 23]. A study conducted by Barker et al., associated low education levels with lack of making healthy food choices, which may contribute to the caries experience[25]. Seventy percent of the ECC group in our study reported only a primary or secondary school education, suggesting that parental education level is associated with early childhood caries.

Self-reported medical history between both groups did not prove to play a role in the caries experience. However, inconsistent sun exposure was reported on the questionnaire. Sun exposure in the form of ultraviolet B radiation has an impact on Vitamin D production in addition to reports of reducing the risk of dental caries. Solar ultraviolet radiation that penetrates our skin is responsible for the conversion of pre-vitamin D_3 to Vitamin D_3[8]. Studies indicate that solar UVB radiation exposure was inversely correlated with dental caries.[28] Although sun exposure increases circulating vitamin D[16], further studies should be done to make a correlation with dental caries status. Our study did not question sun exposure in great detail in order to make a definitive relationship.

Increased consumption of sucrose, frequent snacking and sugary drinks throughout the day, is a risk factor for early childhood caries[21]. A classic study conducted from 1945-1952 in a Swedish mental institution provides data on the association between sugar consumption and

dental caries that is still relevant today. The named Vipeholm study made a strong correlation between the frequencies of consuming sugar versus the amount of sugar consumed playing an active role in the etiology of caries [26].We also found an association between frequent snacking and increased intake of sugary drinks in the development of caries in the ECC group. Almost half of the ECC group (n=4/10) reported having a bottle in the bed and more than half reported still using a bottle or sippy cup (n=6/10). Although not solely implicated in caries, frequent nighttime bottle-feeding with milk is associated with ECC and has been found to be cariogenic in conjunction with other carbohydrates. [17]

Where parental caries experience was reported, our findings indicated that parents of the ECC group had a higher caries experience compared to the controls. This data is in line with previous studies that indicate that a child is at higher caries risk if the parents have a history of caries. [23,24]Transmission of the infectious organism involved in the caries process, *Mutans streptococci*, may occur between parent and child at an early age, causing colonization of the tooth surfaces.[20]

Data from our study is consistent with previous studies suggesting that children with early childhood caries and severe early childhood caries (defined as smooth surface caries in children younger than 3 years old)[22] is associated with low levels of Vitamin D and elevated levels of PTH.[10] In a similar pilot study, 38 children participated, 50 percent of whom had severe early childhood caries. Serum samples of the children with S-ECC who were undergoing dental surgery as well as caries free children were obtained and analyzed for Vitamin D and parathyroid (PTH) hormone levels. Although a majority of the sample size had inadequate Vitamin D levels, children with S-ECC had lower concentrations of 25(OH) D and higher PTH levels than caries free children.

Eighty percent of the ECC participants in our study were Vitamin D deficient compared with the control group. Parathyroid hormone and calcium levels were also compared between the two groups. Physiologically PTH and vitamin D are inversely related. When Vitamin D levels decrease, PTH acts as a regulatory hormone and increases to conserve calcium. [7, 27] Conversely, serum calcium levels are normal in vitamin D deficient children. [27] We found positive correlations found between vitamin D and calcium between both groups to support this.

As a pilot study, data was limited based on the sample size. Future studies are aimed at increasing the subject population to support the data currently obtained. Increasing the sample size will allow enough data to correlate findings with our hypothesis. Multiple covariables exist that also contribute to caries. Our aim was to measure and assess those variables as they related to vitamin D deficiency and caries. Through our questionnaire, dietary habits, oral hygiene habits and socioeconomic and sociodemographic factors were obtained. However, we found that many questions were left unanswered and could have been formed more clearly for the parent. More specifically, questions about fluoride in the water and sun exposure seemed to be unclear.

In addition to the early studies correlating vitamin D and caries, studies were also conducted to determine the relationship between sun exposure and the incidence of dental caries. It was found that caries incidence was inversely related to hours of sunshine and geographic location.[30,31] Our questionnaire did not specify length of sun exposure nor did we take into consideration the amount of sun exposure the patients received during the time of year the blood was drawn. Further continuation of the study will include a modification of the questionnaire to include determination of hours of sun exposure in both study populations.

Lack of sufficient population in the groups in this study may have also introduced a source of bias. Our aim was to obtain age matched controls, however, the feasibility of obtaining

control patients was limited by the frequency of patients coming in for well child exams in which blood samples were being obtained. Institutional Review Board (IRB) approval was obtained on March 25, 2014, for a second method of obtaining control patients for continuation of the study. Healthy, caries free children who meet the inclusion criteria and will be undergoing ear, nose and throat procedures under general anesthesia will be considered as controls. Our goal is to have controls that are similar to the ECC group for comparison. The age range of the control group was younger (age 1 year and 19 months) and these children had fewer teeth than the ECC group. There also exists a difference in the exposure time of the teeth to the oral environment as well as time for formation of poor eating and oral hygiene habits.

Future studies are aimed at obtaining sufficient sample size and obtaining controls that are similar in age and demographics to the ECC group. Modifications in the existing questionnaire will be made such that parental comprehension is increased and all questions are answered. Results from continuation of this pilot study should be used to counsel parents not only of the role that bacteria and hygiene play in the development of caries, but to inform them of the importance of minerals, such as vitamin D, in the prevention and cessation of early childhood caries.

Conclusion

Children in the ECC group were found to have insufficient and deficient levels of 25(OH)D. The mean levels of PTH in some of the children in the ECC group were also found to be elevated, which may result from decreased levels of 25(OH)D. Insufficient and deficient levels of 25(OH)D may be associated with the development of ECC. A study with a larger sample size represented is necessary to make a definitive correlation between the two.

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Tables

Table 1. Demographic Characteristics of the Participants (n = 12)

Characteristic	Count	
	Control	ECC
Race		
AA	0	4
AA, Native Hawaiian or Pacific Islander	0	1
White	0	4
White, AA	0	1
White/Caucasian	2	0
Education level		
elementary and middle school	0	1
high school	0	6
college/trade school	0	1
College	1	1
graduate school beyond college	1	1
Parent employed		
Yes	2	6
no	0	3
Household Income level		
less than 5,000	0	3
10,000-19,999	0	1
20,000-29,999	0	1
50,000-79,999	0	2
100,000 or more	2	1

Table 2. Medical History of the Participants

Characteristic	Count	
	Control	ECC
Medical Problems		
Respiratory disorder (asthma)		2
Heart condition		
Neurological disorder (seizures)		
ADHD/ADD		1
Problems with eyes, ears, nose or throat		
Premature birth (more than 3 weeks before the expected date)		1
Illness or infection as a newborn		1
Blood disorder (Sickle cell anemia, hemophilia)		
Genetic disorder/syndrome		
Other		1
None	2	5
Medications		
no	1	6
vitamin D	1	0
vitamins	0	1
yes	0	1
Sun Exposure		
daily	1	4
weekly	0	3

Table 3. Dental History

	Count	
Characteristic	Control	ECC
Child's teeth or tooth care		
Fluoride in water		
yes	1	3
no	0	2
don't know	0	2
Fluoride supplement		
yes	1	1
no	1	8
don't know	0	1
Fluoride treatment		
yes	0	5
no	2	4
don't know	0	1
Brushing frequency		
daily	1	9
weekly	0	1
never	1	0
flossing frequency		
daily	0	4
never	2	6
Parent and child's dental care		
Dental visits		
twice a yr	0	8
yearly	0	2
never	2	0
Difficulty getting to dentist		
yes	0	2
no	2	8
Parent caries		
yes	0	7
no	2	3
Parent have natural teeth		
yes	2	10
Parent brushing		
daily	2	10
Medicaid		
yes	0	8
no	2	2
Parent insurance		
yes	2	6
no	0	4

	Count	
Characteristic	Control	ECC
Public assistance		
yes	0	4
no	2	6

Table 4. Child's Eating Habits

	Count	
Behavior	Control	ECC
Bottle/ sippy cup		
yes	1	6
no	1	4
Bed w/bottle		
never	1	6
weekly	1	0
daily	0	4
Snacks before bedtime		
never	1	2
monthly	0	1
weekly	0	2
daily	1	4
Snacks throughout the day		
never	1	0
weekly	0	1
one or two times a day	1	7
three times or more a day	0	2
sugary drinks		
one or two times a day	0	5
weekly	0	1
monthly	1	0
never	1	0
three or more times a day	0	4
Tap water		
daily	1	5
weekly	1	1
never	0	4

Table 5. Serum Vitamin D Results

Group	n	Mean	SD	SE	P-value
Control	2	39.90	10.47	7.40	
ECC	10	24.96	10.78	3.41	
Difference		-14.94		8.33	0.1029

	n		
	L	N	
Control	0	2	
ECC	8	2	0.0217

Table 6. PTH Results

Group	n	Mean	SD	SE	P-value
Control	2	43.70	18.81	13.30	
ECC	10	54.68	24.71	7.81	
Difference		10.98		18.73	0.5707

	n			
	L	N	H	
Control	0	2	0	
ECC	1	6	3	0.4033

Table 7. Calcium Results

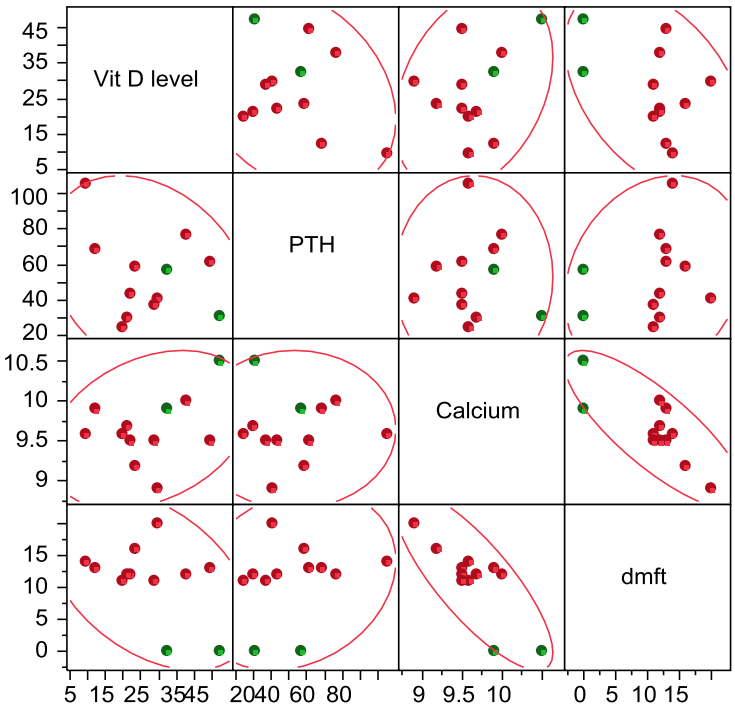
Group	n	Mean	SD	SE	P-value
Control	2	10.20	0.42	0.30	
ECC	10	9.54	0.32	0.10	
Difference		-0.66		0.26	0.0270

	n	
	L	N
Control	0	2
ECC	1	9

Table 8. Relationships between Measurements

Pair		Correlation	P-value
PTH	Vit D level	-0.29	0.3650
Calcium	Vit D level	0.32	0.3049
Calcium	PTH	0.01	0.9875
dmft	Vit D level	-0.43	0.1606
dmft	PTH	0.22	0.5014
dmft	Calcium	-0.81	0.0015

Figure 1. Relationships between Measurements



Appendices

Questionnaire for the primary caregiver

We are conducting a study about the role of Vitamin D deficiency in the development of dental cavities in children. Please select the best answer to the following questions. Thank you.

These basic questions are about your child's age and background.	
How old is your child?	Age: _____
What is your child's racial background? (check all that apply)	☐ White/Caucasian ☐ African American or Black ☐ Asian ☐ Native Hawaiian or Pacific Islander ☐ American Indian or Alaskan Native ☐ Other (please specify)

Please help us understand your child's medical history (Select all that apply to your child)	
☐ Respiratory disorder (asthma)	☐ Premature birth (more than 3 weeks before the expected date)
☐ Heart condition	☐ Illness or infection as a newborn
☐ Neurological disorder (seizures)	☐ Blood disorder (Sickle cell anemia, hemophilia)
☐ ADHD/ADD	☐ Genetic disorder/syndrome
☐ Problems with eyes, ears, nose or throat	☐ Other_____
Does your child take any medications or vitamin supplements? If so please list them.	Yes No Don't know _____
How often is your child exposed to the sun?	Daily Weekly Monthly Never
Is there fluoride in your drinking water at home?	Yes No Don't know

The first few questions are about your child's teeth	**(Select one)**
1. Does your child have any cavities or fillings?	Yes No Don't know
2. Did your child's doctor or dentist prescribe fluoride drops or tablets?	Yes No Don't know
3. Does your child receive fluoride painted/put on their teeth from a health professional (doctor, dentist, nurse, hygienist, etc.)?	Yes No Don't know

Now we want to ask about your child's tooth care	**(Select one)**
4. How often does an adult brush your child's teeth with toothpaste?	Daily Weekly Monthly Never
5. How often are your child's teeth brushed with non-fluoride toothpaste?	Daily Weekly Monthly Never
6. How often are your child's teeth flossed?	Daily Weekly Monthly Never

Next we ask about your child's eating habits	**(Select one)**
7. Does your child usually (throughout the day) drink from a bottle or sippy cup?	Yes No

8. How often does your child go to sleep while nursing or while drinking something other than water from a bottle/sippy cup?	Daily Weekly Monthly Never
9. How often does your child eat or drink anything other than plain water before going to bed or after you have brushed his/her teeth?	Daily Weekly Monthly Never
10. How often do you give your child sugary snacks such as raisins, candy, cookies, cakes, or cereal between meals?	☐ Three or more times a day ☐ One or two times a day ☐ Weekly ☐ Monthly ☐Never
11. How often do you give your child sugary drinks such as regular soda, sweet tea, chocolate milk, strawberry milk or fruit juice between meals?	☐ Three or more times a day ☐ One or two times a day ☐ Weekly ☐ Monthly ☐Never
12. How often does your child typically drink tap water- including filtered water from the refrigerator?	Daily Weekly Monthly Never

The following questions are about you and your child's dental care	**(Select one)**
13. How often do you take your child to the dentist?	☐ Never ☐ Only when in pain ☐ Yearly ☐ Twice a year
14. Is it very difficult to get your child to the doctor or dentist?	Yes No
15. Is your child's care covered by Medicaid or State Insurance?	Yes No Don't Know
16. Is your child covered by any health or dental insurance other than/or in addition to Medicaid or State Insurance?	Yes No Don't Know
17. Does your child participate in public assistance programs (ex: WIC, Healthy Start, etc.)?	Yes No Don't Know

Now we will ask about your teeth and your tooth care	**(Select one)**
15. Do you have cavities or fillings or have had teeth pulled in the last 2 years?	Yes No
16. Do you have any of your own natural teeth	Yes No
17. How often do you brush your teeth?	Daily Weekly Monthly Never
18. Do you have dental insurance?	Yes No
19. How often do you get dental checkups?	☐ Never ☐ Only when in pain ☐ Yearly ☐ Twice a year

Now tell us a little bit about you…	
20. What is the highest level of education that you completed?	☐ Elementary and Middle School ☐ High School ☐ College ☐ Graduate school beyond college
21. Is an adult in the child's household employed?	Yes No

22. Which of the following categories best represents the combined income of all family members in your household for the past 12 months? (select one)	□ Less than $5,000 □ $5,000-$9,999 □ $10,000-$19,999 □ $20,000-$29,999 □ $30,000-$39,999 □ $40,000-$49,999 □ $50,000-$79,999 □ $80,000-$99,999 □ $100,000 or more □ Don't know

Thank you so much for answering these questions. This information will better help us to learn more about the relationship between vitamin D deficiency and children's dental health.

Vita

Tiffany Lasean Williams was born on July 22, 1982, in Richmond, Virginia and is an American citizen. She graduated from Richmond Community High School, Richmond, Virginia in 2000. She received her Bachelor of Science in Biology from Spelman College, Atlanta, Georgia in 2004 and subsequently attended a post baccalaureate program at Southern Illinois University, Carbondale, Illinois for two years. She received a Doctorate of Dental Surgery from The University of North Carolina Chapel Hill in 2010.

After graduation, she served as a General Practice Resident at Carolinas Medical Center in the Department of Oral Medicine, Charlotte, North Carolina. She completed a Chief Resident year at Jabobi Medical Center in the Department of Dentistry, Bronx, New York. Tiffany is currently a second year resident in the Department of Pediatric Dentistry at Virginia Commonwealth University, Richmond, Virginia and will be earning a specialty certificate in pediatric dentistry upon graduation.