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A Comparison of Vitamin D Levels in Children with Early Childhood Caries

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A Comparison of Vitamin D Levels in Children with 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

A COMPARISON OF VITAMIN D LEVELS IN CHILDREN WITH EARLY CHILDHOOD CARIES

By Vanessa Ong Hofileña, DDS

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Major Director: Tegwyn Brickhouse, DDS, Ph.D.
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Purpose: To determine if there is an association between early childhood caries (ECC) and vitamin D deficiency, as measured via a serum sample. An exploratory goal was to investigate the possibility of measuring vitamin D with a salivary assay. **Methods:** Serum samples of patients who were scheduled for a dental or otolaryngology procedure under general anesthesia were assessed for 25-hydroxyvitamin D (25(OH)D), parathyroid hormone and calcium. **Results:** Our analysis indicates that the vitamin D levels of the controls and ECC group were significantly different. There were significant inverse correlations between: 1) PTH levels and vitamin D and 2) decayed, missing, filled teeth (dmft) and vitamin D. **Conclusions:** A relationship between low serum levels of vitamin D and the prevalence of ECC for children 0 to 6 years of age

was observed. The salivary assay was unable to measure vitamin D, but future studies should still explore this non-invasive technique.

Introduction

While obesity and asthma may be at the forefront of the national dialogue on children's health, dental caries is considered the most chronic childhood disease.[1] According to the American Academy of Pediatric Dentistry, early childhood caries (ECC) is defined as the occurrence of one or more decayed, missing, or filled surfaces in the primary tooth of children below six years of age.[2] Currently, dental caries is four times more common than childhood obesity, five times more common than asthma, and 20 times more common than diabetes.[1] Yet, due to the increasing problem of limited access to dental care, the vast majority of dental caries in children is still left untreated; this unfortunate situation results in countless deleterious effects to a child's overall well-being.[3] Untreated dental caries can adversely influence a child's self-esteem, food intake, sleep, and overall quality of life.[3] Due to the rising incidence of dental caries in children, contributory factors need to be investigated in order to facilitate the treatment of such a large population of children in desperate need of dental care.[3] These multiple risk factors include the lack of a dental home, poor oral hygiene, diet, and nutrition.[4] Recent studies have found evidence suggesting a correlation between early childhood caries and vitamin deficiency, specifically a deficiency in vitamin D.[5]

Vitamin D, a fat soluble vitamin, is considered to be one of the oldest hormones that exist, with the most primitive forms of life possessing the ability to convert sunlight into vitamin D_2.[6] It is nicknamed the "sunshine vitamin" because it is obtained primarily from ultraviolet light and absorbed through the skin.[6] Holick et al showed that when the skin is exposed to 1 minimal erythemal dose (MED) of ultraviolet sunlight, around 20,000 units of vitamin D is produced.[7] Vitamin D can also be acquired through a few foods sources such as fatty fishes (salmon, tuna, mackerel), fish liver oils, cheese, beef liver, and egg yolks or directly by dietary supplementation. Despite the availability of vitamin D in some foods, regular exposure to vitamin D from sunlight remains to be the most effective mechanism in which to obtain and prevent vitamin D deficiency in both infants and young children.[7]

As illustrated in Figure 1, vitamin D is comprised of the following compounds: vitamin D_3 (cholecalciferol) and vitamin D_2 (ergocalciferol).[8,9] Specifically, vitamin D_3 is formed after the conversion of its precursor molecule, 7-dehydrogenase cholesterol. Vitamin D_3 is absorbed into the body when ultraviolet B wavelengths between 270-300nm contact the skin (especially in the summer) or from the consumption of animal food sources.[8-12] Vitamin D_2 is obtained from plants or plant derivatives. Vitamin D_2 and vitamin D_3 are biologically inert and are activated via two hydroxylation reactions.[9] The first hydroxylation reaction occurs in the liver which forms 25-hydroxyvitamin D (25(OH)D or calcidiol); this is the major circulating form of vitamin D and is the primary form of vitamin D used to determine a patient's vitamin D status.[7,9,13] Its half-life ranges from 2-3 weeks, so it is a reliable indicator of an individual's measure of dietary and ultraviolet vitamin D over long periods of time.[14] The second hydroxylation reaction occurs in the kidney, where the most biologically active metabolite 1, 25-dihydroxyvitamin D (1, $25(OH)_2D$ or calcitriol) is formed; 1,25-dihydroxyvitamin D has a half-life of approximately two and half hours.[9,13-15] 1, 25-dihydroxyvitamin D is responsible for the absorption of calcium and phosphorous in the intestine and kidney. When vitamin D levels are low, calcium is not adequately absorbed and the body releases parathyroid hormone (PTH) to stimulate the mobilization of vitamin D from skeletal tissues.[16] It is through this homeostatic mechanism that plasma parathyroid hormone and serum calcium and phosphorous levels provide control over renal production of 1, 25-dihydroxyvitamin D.[14] Another main regulator of 1, 25-dihydroxyvitamin D is fibroblast growth factor 23; this works to suppress the synthesis of 1, 25-dihydroxyvitamin D by internalizing the sodium-phosphate co-transporter in the kidney and small intestine.[14] Concurrently, these mechanisms work together to maintain vitamin D homeostasis within the body.

According to Holick, there are approximately one billion people worldwide who suffer from either vitamin D deficiency or insufficiency.[17] In the United States and Europe alone, 40 to 100 percent of the elderly population and 50 percent of postmenopausal women who are diagnosed with osteoporosis, are considered to be vitamin D insufficient. Some predictors for an imminent vitamin D insufficiency or deficiency diagnosis in children include the season (specifically winter), being non-Caucasian or female, obesity, low milk consumption, low socioeconomic status, having a more advanced stage of puberty, or less time spent outdoors.[8] Of these risk factors, seasonal variability has the greatest influence on vitamin D status. Vitamin D levels decline significantly during the winter months, when compared to the summer, spring, or autumn seasons. From these findings, it may be beneficial for more clinicians to recommend vitamin D supplementation and increased sunlight exposure to those children and adolescents who are found to have lower levels of vitamin D.

Although no true agreement has been made regarding an exact definition of vitamin D deficiency or what levels of vitamin D are optimal for health, the majority of experts concur that vitamin D deficiency occurs when the 25-hydroxyvitamin D (25-OH) level is less than 20ng per mL (50nmol/L).[8,18] Individuals displaying 20-30ng per mL (50-75nmol/L) are considered to be vitamin D insufficient, whereas those who have serum levels greater than 30ng per mL (75nmol/L) are deemed to be vitamin D sufficient. Vitamin D levels up to 100ng per mL (250 nmol/L) are considered safe levels for children and adults (Table 1 and Figure 2). Once these levels reach unsafe serum concentrations of more than 150ng per ml (375nmol/L), vitamin D toxicity occurs. Several symptoms of vitamin D toxicity in children include the following: poor appetite, constipation, polydipsia, polyuria, abdominal pain, vomiting, and in extreme cases, individuals may even develop severe dehydration which could potentially be life threatening.[19]

Vitamin D is responsible for maintaining normal calcium and phosphate levels in blood and is integral to both bone and mineral metabolism that is required for the normal calcification of bone growth plates.[8] When vitamin D levels are deficient, only 10-15 percent of dietary calcium and 50-60 percent of dietary phosphorous can be utilized by the body.[14] Due to this, deficiencies in this vitamin contribute to the manifestation of several metabolic bone diseases such as osteomalacia, osteoporosis, and rickets.[8,20] Growth retardation and skeletal deformities observed in children are termed osteomalacia, whereas it is referred to as osteoporosis when displayed in adults.[8] Clinically in osteomalacia, the trabecular and cortical surfaces of bone are surrounded by thick osteoid seams; this differs from osteoporosis, where only small amounts of osteoid are apparent.[10] Osteoporosis is defined as a systemic skeletal disease which exhibits low bone mass, deterioration of bone tissue, and an increase in both bone fragility and the incidence of fractures.[21] The prevalence of osteoporosis increases significantly with age, with 30-40% of adults over 60 years of age diagnosed with the disease. Other risk factors for osteoporosis include poor nutrition, genetics, menopause, medications, and chronic illness. Another manifestation of vitamin D deficiency, rickets, is observed before a child is 18 months of age, with the majority of children being diagnosed between four and 12 months.[14] Some particular examples of skeletal deformities as a result of rickets include: inward or outward tibial and femoral bowing, chest deformation, involution of the ribs and protrusion of the sternum (termed "pigeon chest"), and hypertrophy of the costochondral junctions causing beading (rachitic rosary appearance).[22] In 1919, there was evidence showing that ultraviolet exposure from a mercury arc lamp caused an improvement in severe rickets.[22] As a result of these findings, it is evident that vitamin D is fundamental to good health and proper bone development.

In addition to the negative sequelae of vitamin D deficiency related to impaired bone metabolism, a deficiency in vitamin D can also be deleterious to an individual's overall health; this can ultimately result in the improper functioning of the body's immune system and impaired healing.[20] Specifically, maintaining healthy levels of vitamin D has been proven to minimize the risk of autoimmune disease, cancers, infections, obesity and diabetes.[23] A randomized controlled study conducted in Japan found that administering vitamin D supplementation to school children during the winter contributed to a decrease in the frequency of influenza outbreaks.[24] There is also compelling evidence supporting a relationship between vitamin D insufficiency or deficiency with respiratory diseases such as asthma. Another study conducted by Lewis et al. found that a lower serum concentration of 25(OH)D was observed in asthmatic children, whereas a randomized controlled study by Majak et al. demonstrated that the risk of an asthmatic attack as a result of an acute respiratory tract infection was reduced when vitamin D supplements were taken daily.[25,26] Similarly, obesity, whose prevalence is increasing worldwide, has also been implicated in vitamin D deficiency.[27] The relationship between obesity and vitamin D deficiency may be attributed to increased levels of the “starvation hormone,” leptin, in obesity, which inhibits the renal synthesis of active vitamin D and sequesters vitamin D in body fat; additionally, children suffering from obesity may not spend as much active time outdoors and thus, have lower levels of vitamin D due to decreased sun exposure.[28-30] Collectively, these research conclusions demonstrate how our body depends considerably on the maintenance of proper vitamin D levels.

Not only does vitamin D contribute to overall health, it also plays a key role in regulating craniofacial development and ensuring optimum oral health.[5] The “sunshine vitamin” is indispensable in promoting the calcification of teeth, having a topical fluoride-like effect, and in the forming of enamel, dentin, and bone.[31-33] A deficiency in vitamin D levels adversely influences tooth development by producing marked developmental defects, such as enamel hypoplasia, which has been identified as a risk factor for S-ECC.[5] In an infant based study, investigators exhibited that prenatal supplementation with vitamin D decreased the occurrence of enamel defects such as enamel hypoplasia.[34] Another infant based study revealed that there was a correlation between the absence of vitamin D supplementation and delayed tooth eruption.[34] There is also compelling evidence suggesting a link between low serum levels of vitamin D to both caries and periodontal disease. Specifically, the findings from a study performed by Schroth et al., which evaluated the relationship between serum concentrations of vitamin D and S-ECC in pre-school children, demonstrated that children with S-ECC had lower vitamin D levels (25(OH)D) and higher PTH levels in comparison to caries-free controls.[5] There has also been research suggesting that ameloblasts and odontoblasts are target cells for 1,25 dihydroxyvitamin D and that vitamin D plays an immunological role by protecting us from oral pathogens through the production of antimicrobial peptides (cathelicidin and certain defensins).[5] The findings of a historical study conducted by Mellanby in the 1920's also suggested that vitamin D was important in the development and calcification of enamel and dentin.[33] This study validated that in those children who consumed a high vitamin D diet, vitamin D played an indispensable role in 1) preventing the initiation of new caries, 2) inhibiting the spread of existing caries, and 3) arresting the caries.[33] Likewise, an examination of children by East found a decreased caries prevalence in areas of increased vitamin D exposure.[35] East discovered that vitamin D levels were highest in those areas that received the greatest amount of sunshine and during the months of greatest sunshine.[35] These findings illustrate the importance of examining a child's serum vitamin D level in conjunction with his or her oral health and the value of implementing vitamin D supplementation when needed, to assist in decreasing the occurrence of dental caries.

The discovery of the significance of vitamin D in promoting overall health has encouraged the need to explore simple, non-invasive methods to assess vitamin D levels in human bodily fluids. Besides its protective antibacterial qualities and essential role in preserving oral health, saliva serves as a valuable biofluid, and has been proven to be beneficial in both laboratory and clinical diagnosis or in overseeing patients with oral or systemic conditions.[36] Advantages of using saliva over blood samples for diagnosis include: (a) it is a non-invasive and rapid technique that permits the collection of several samples; (b) collecting saliva is effortless and painless; (c) collection does not require extensive training; and (d) saliva poses only a minimal risk for spreading infection.[37] At this time, the most frequent and accurate mode utilized to measure the levels of vitamin D is through serum samples. A study conducted by Higashi et al. demonstrated that 25-hydroxyvitamin D_3 levels are reliably measured by unstimulated saliva, which offers an innovative, non-invasive, and non-stressful alternative to measuring vitamin D levels.[13,38] Developing reliable salivary assays that measure vitamin D can be monumental in detecting 25-OH vitamin D levels in children.

Currently, no studies have specifically assessed vitamin D levels in both the serum and saliva in children with early childhood caries. An exploratory goal of this study is to determine if vitamin D levels can be measured in saliva samples of children. Our specific aims are:

- To determine if there is a group difference between the following serum values: vitamin D, parathyroid hormone (PTH), and calcium.
- To determine the correlation between the following: serum vitamin D, serum PTH, serum calcium, and the decayed, missing, filled teeth score (dmft).

Research Design and Methods

This is a cross-sectional pilot study with cases and controls investigating the usefulness of saliva and serum in detecting caries in children based on a child's vitamin D level.

<u>Inclusion and Exclusion Criteria</u>

To be included or excluded in this study, the following conditions needed to be met:

- Children ages 71 months or younger were approached for participation. Children were only asked to participate in this study if they were classified as ASA 1 (healthy) or ASA 2 (mild systemic disease). The American Society of Anesthesiologists (ASA) physical status classification system is a six-category assessment tool used to determine the fitness of cases prior to surgery.[11]
- Children were excluded from this study if they were older than 71 months, had an ASA classification of ASA 3 or greater, or had a complex metabolic or medical disorder.

<u>Subject Recruitment</u>

There were two groups of patients recruited into this study, cases and controls. Data for this particular study was collected at VCU Health System's operating room and Children's Pavilion from March 1, 2014 to January 1, 2015.

Recruitment of Case Patients

Case patients presented to the Virginia Commonwealth Pediatric Dental Clinic for a new patient dental exam and met the criteria for diagnosis of early childhood caries

(ECC). Early childhood caries is defined as the existence of one or more decayed (cavitated or non-cavitated), missing (from caries), or filled tooth surfaces in a primary tooth of a child six years old or younger (dmft). The dmft was recorded for each patient after a calibrated clinical exam was performed. Those patients who met the inclusion criteria for the study, and were scheduled for full mouth dental rehabilitation (FMDR) under general anesthesia were asked to participate in the study. Caregivers were not made cognizant of this study during this initial appointment, so this study did not influence their decision to pursue full mouth dental rehabilitation under general anesthesia. The majority of the caregivers chose this treatment option due to one of the following reasons: complex dental treatment needs of the child, difficulties with transportation or long distances traveled for dental appointments, or behavioral obstacles that prohibited the successful completion of dental treatment in the office.

The morning of the scheduled dental surgery, the caregivers of the case patients were approached in the peri-operative area by a trained Virginia Commonwealth University Pediatric Dental Resident who was familiar with the study. After thoroughly explaining the goals of the study in both written and verbal forms to the caregiver, informed consent was acquired. All caregivers were informed that the risks of participating in the study were low, since the child was already receiving an intravenous line and thus, would not incur an additional needle-stick. All caregivers were given the opportunity to ask questions and withdraw from the study at any time.

Caries-Free Control Patients

Caries-free control patients were recruited by one of the two methods described below.

During initial data collection, two control subjects were recruited in a primary care setting during their annual well-child checks. The remaining dental caries-free controls were recruited from healthy pediatric patients that were scheduled for an Ear, Nose, and Throat (ENT) surgical procedure with the VCU otolaryngology service. Controls were approached and recruited in the same manner as those patients undergoing full mouth dental rehabilitation under general anesthesia. A calibrated visual exam with a plastic mirror and light was completed at the time of recruitment to ensure that no frank, cavitated lesions were present. Only those patients who were caries-free and met all the inclusion criteria were asked to participate. Additionally, only children undergoing ENT surgical procedures that required an intravenous line (adenoidectomy, tonsillectomy, etc.) were requested to participate, so that no additional needle-stick was incurred while participating with this study. Similar to the procedure followed with the caries patients, informed consent was acquired after explaining the goals of the study and all caregivers were given the opportunity to ask questions and withdraw from the study at any time.

Questionnaire

A demographic and behavioral questionnaire was administered to all caregivers who agreed to participate in the study (Appendix A). This questionnaire was essential in ascertaining additional biological and non-biological risk factors that could be influential in the presence of caries or current vitamin D levels. The questionnaire included questions regarding: both 1) demographic characteristics and 2) behavioral characteristics (i.e., dental and dietary habits of the child and caregiver).

Blood Samples

Serum samples were obtained by the anesthesia team while the patient underwent general anesthesia preceding the commencement of their dental or ENT surgery. After anesthesia personnel collected approximately seven milliliters of blood, the samples were divided into two different collection tubes: a gold capped BD Vacutainer tube that held a maximum of 4.0 ml of blood and a violet capped BD Vacutainer tube that stored a maximum of 3.0 ml of blood and contained around 5.4 mg of EDTA. After the blood samples were distributed into the BD Vacutainer collection tubes, they were placed into a clear biohazard specimen transport bag and were immediately transported to VCU Hospital's Clinical Pathology Laboratory for analysis. All samples were given a unique Research Medical Number in the Cerner Hospital database in order to ensure that all samples were accounted for and properly analyzed.

Blood samples obtained from caries-free controls during their annual well child exams were obtained by a clinical nurse assigned to the patient's care. These samples were also stored and assigned a research identification number in the same manner.

Serum Analysis for Vitamin D

Blood analysis for vitamin D levels were conducted by the VCU Hospital Health Systems Clinical Pathology Laboratory team using liquid chromatography and tandem mass spectroscopy. The sum of total 25-OH vitamin D_3 and 25-OH vitamin D_2 were calculated to establish a measurement for the total 25-OH vitamin D, which has been utilized by the National Institute of Technology as a SRM 972a serum reference material for 25-OH vitamin D.[39] The data was recorded in a database and charted for data analysis. Additionally, parathyroid hormone (PTH), calcium, and 25(OH)D levels were measured in the same blood sample. PTH is a more sensitive surrogate for vitamin D deficiency and is usually analyzed together with vitamin D. Analysis for our data defined and classified vitamin D based on the following acknowledged values: <20ng/mL for deficiency, 20-30ng/mL for vitamin D insufficiency, >30ng/ml for vitamin D sufficiency (Table 1 and Figure 2) [8,29]. Values for parathyroid hormone and calcium were also further classified into low, normal, or high values based on pediatric averages outlined by Virginia Commonwealth University's Department of Pathology Laboratory. According to their guidelines, the following values defined low, normal, or high: 1) low is <12.0pg/mL, normal is =12.0-65.0pg/mL, high is >65.0pg/mL for parathyroid hormone and 2) low is <9.1mg/dL, normal =9.1-10.9mg/dL, and high is >10.9mg/dL for calcium (Table 1 and Figure 3).

After receiving the results, all caregivers were immediately contacted via telephone to make them aware of the vitamin D status of their child. If vitamin D deficiency or insufficiency was discovered, the patient was referred for an evaluation to their pediatrician.

Analytical Plan

The responses to the demographic questionnaire and serum levels were entered into a Redcap database and analyzed using Statistical Analysis Software (SAS Institute Inc., Cary NC) at alpha=0.05. Analysis included descriptive statistics (frequencies relating to demographics, dmft scores, vitamin D and PTH levels and mean +/- SD values). A two group t-test was conducted to see if there were differences in the serum vitamin D, PTH, and calcium levels of the ECC group versus the controls, while a chi-square analysis was performed to determine if there were any differences between the groups when the values were classified as either: low, normal, or high. Pairwise correlations were used to determine correlations between the serum levels (vitamin D, PTH, and calcium) and the decayed, missing, and filled teeth score (dmft). For the questionnaire, a chi-square analysis was used to determine differences in the following: income, education, and dietary habits (frequency of bottle use, snacking, sugary drinks, and fluoride water consumption). Finally, a Fisher's exact test was used to analyze if any differences between the two groups exist in race or ethnicity.

Results

A total of 56 questionnaires were returned from the cases and controls, with 2 surveys from the ECC group not collected from the caretakers due to time constraints the morning of their child's surgical procedure.

<u>Results of the caregiver questionnaire</u>

Subjects

A total of 58 children were enrolled in the study. There were thirty-eight children (cases) with early childhood caries and 20 children who were determined by visual exam as caries-free. The mean age for the controls was 37.5 months (SD±18.15 months), whereas the mean age for the children with early childhood caries (ECC) was 50.8 months (SD±13.90 months). Table 2 shows the mean age of the participants recruited for this study. The control children were significantly younger than the case children ($p=0.0028$). Ethnicity also varied widely amongst the participants, as demonstrated in (Table 2). There was a statistically significant difference in ethnicity, with the ECC group having more self-identify as African American ($p=0.0135$) and the control group having more Caucasian children ($p=0.0119$). The caregivers also reported a wide range of medical health histories on behalf of their children (Table 3). The caregiver responses regarding their child's sun exposure is presented in Table 3. In general, control children had more daily sun exposure (86%) compared to ECC children (79%).

Dietary Status of the Child

The dietary habits of the child are displayed in Table 4. According to Table 4, more than half of both groups did not use a bottle or sippy-cup (58% of controls and 66% of ECC children), and there was no statistically significant difference between the two groups regarding bottle or sippy-cup use (p=0.5699). A considerable amount of controls (95%) did not bring a bottle to bed when compared to ECC patients (78%), although bottle use during bedtime was not statistically significant (p=0.1710). Fourteen percent of children with caries brought a bottle to bed daily, while 8 percent brought one to bed weekly. Ninety percent of healthy children never ate anything before bedtime; contrastingly, only 41 percent of the ECC group did not have a snack before bedtime and 56% of ECC children had snacks before bedtime daily. A large percent of children in both groups had daily snacks throughout the day (86% in the ECC group, 60% in the control group). Ten percent of the control samples never had snacks throughout the day, with 30 percent of controls and 14 percent of the ECC group having a weekly snack. Most of the children in both groups drank some form of a sugary drink daily (63% of control versus 81 percent in ECC children), with 21 percent of control children never having a sugary drink. Approximately 11 percent of controls and 17 percent of ECC children had a sugary drink weekly. Eighty percent of the controls and 61 percent of ECC patients had tap water daily, whereas 15 percent of controls and 22 percent of the ECC group never drank tap water. According to the chi-square analysis, there was a statistically significant difference between the two groups in both snacking and sugary drink consumption. Significantly more of the ECC group had snacks before bedtime and throughout the day (p=0.0120 and p=0.0063, respectively); additionally, a statistically greater number of ECC children consumed sugary drinks more frequently (p=0.0151). The analysis did not show a statistical significance in the frequency of tap water consumption between the two groups (p=0.2986).

Dental Status of the Child

Table 5 describes the dental history of the child. A greater number of control children lived in an area with fluoridated water (68%) compared to those who belonged to the ECC group (48%), with a larger percentage of ECC caregivers who didn't know if there was fluoride in their water (22% versus 5% in the control group). The vast majority of both groups responded that their child did not take any additional fluoride supplements (85 percent of the control versus 77 percent of the ECC group). When asked if their child received fluoride treatment from a health professional, the majority of control patients (60%) replied "no" and 35% replied "yes." Similar percentages of ECC caregivers said that their child either received fluoride or did not receive fluoride from a health professional (46% said "yes" and 43% said "no."). The brushing frequency for the children of each group was also assessed. Around 83 percent of ECC caregivers reported that their child brushed their teeth daily, while 90 percent of control children performed daily brushing. A greater fraction of control caregivers (10%) stated that their child never brushed, while only 6 percent of control caregivers never brushed also. Most children in both groups claimed they never flossed (56% in the control and 53% in the ECC group).

Approximately 60 percent of ECC children visited their dentist twice a year, while only 50 percent of healthy children did. A substantial amount of healthy children had never been to the dentist (40%), whereas all the ECC children had been to the dentist whether yearly (31%) or only when in pain (9%). Remarkably, all the caregivers of the control children answered that they had no difficulties getting to the dentist, while 25 percent of the ECC children did. Around 80 percent of the ECC children and 45 percent of the control patients had Medicaid.

Demographics of the caregivers

The demographic characteristics of the caregivers are presented in Table 2. Most of the caregivers in the control group finished educational requirements beyond high school, with 50 percent earning a college degree and 20 percent completing graduate school. It is interesting that all control caregivers earned an education beyond high school, whereas more than half of the caregivers in the ECC group only finished a high school level education or less (61%). A decreased proportion of the ECC caregivers received a college or graduate school degree (28% and 11% respectively) when compared to the control group. In regards to employment status, approximately 84 percent of the control caregivers and 76 percent of the ECC caregivers were employed. Roughly 79 percent of the caregivers in the control group had a household income of $50,000 or more, with 21 percent claiming they had an income of less than $30,000. A greater ratio of ECC caregivers had a total household income less than 30,000 (62%), while only 21 percent earned more than $50,000 per year and 17 percent saying they earned between $30,000-49,999. Although there was no significant difference between the two groups on education level (p=0.0830) or employment (p=0.7264), there was a significant difference in income level (p=0.0003, ECC mean= $31,265 vs control mean = $81,290—calculated using the midpoint of the income ranges).

Dental Status of the caregiver

Table 5 illustrates the dental history of the caregivers. A large proportion of the caregivers claimed they had dental caries (70% of the control, 67% of the ECC caregivers), and all caregivers claimed to have their natural teeth. All caregivers in the control group stated they brushed their teeth daily, while 97 percent of the ECC caregivers completed daily brushing.

Comparison of the groups

This section compares the following levels for each group: vitamin D, PTH, and calcium. As displayed in Table 6 and Figure 4, the mean vitamin D level of the controls was shown to be vitamin D sufficient (32.4 ng/ml, SD±8.57) whereas the mean vitamin D level of the ECC group was vitamin D insufficient (26.6 ng/ml, SD±8.27). The results of our analysis indicate that the difference of the vitamin D levels between the controls and ECC group were statistically significant (p=0.0150). When these numbers were categorized into nominal values (vitamin D deficiency, insufficiency, or sufficiency), the chi-square test suggested a statistical significance (p=0.0455) in the number of vitamin D deficient, insufficient, and sufficient children in each group. Table 7 and Figure 5 demonstrate that a greater number of ECC children were vitamin D deficient (18% of the ECC compared to 0% of the controls) or vitamin D insufficient (53% of the ECC compared to 45% of the controls), and that more control children exhibited vitamin D sufficient levels (55% of the controls compared to 29% of the ECC group).

The final PTH levels are shown in Table 6. Our serum measurements did not show a statistically significant difference in PTH, with a mean PTH level of 47.4 pg/ml (SD±21.34) for the control group and 57.5 pg/ml (SD±28.84) for the ECC group (p=0.1724). Although not significant, the PTH levels in the ECC group were generally higher when contrasted with the control group. Moreover, the analysis did not find a statistical significance when the serum PTH values were categorized into low, normal, and high values (Table 7, p=0.1146). Most of the patients from both groups had a normal PTH value (85% in the control group vs. 61% in the ECC group), with only one child in the ECC group with a low PTH level (Figure 6).

Similarly, the serum measurements of calcium did not demonstrate a statistically significant difference in the calcium levels between the two groups (Table 6, p=0.6673). In particular, the mean calcium level for the control group was 9.6 mg/dL (SD±0.38), while the mean calcium level for the ECC group was 9.6 mg/dL (SD±0.28). The results of the contingency table did not find a statistical significance when the serum calcium values were divided into low, normal, and high values (Table 7, p=0.5920). The majority of children in both groups had normal calcium levels (95% in the control group and 94% in the ECC group), with one child in each group presenting with low calcium values (Figure 6).

The correlations between vitamin D, PTH, calcium, and the decayed, missing, filled, teeth (dmft) score can be seen in Table 8. According to this data, the only statistically significant correlations were between the following: 1) PTH levels and vitamin D (p=0.0030, correlation is r = -0.38), and 2) dmft and vitamin D (p=0.0114, correlation is r = -0.33). Both of the above were inversely correlated. The combined mean dmft for both groups was 7.8 (SD=6.3692), with the control group having an average dmft of 0 and the ECC group having an overall average dmft of 11.9 (Table 9).

Discussion

In the early 1920's and mid 1930's, a unique interest arose to ascertain if vitamin D was beneficial in preventing and arresting the spread of caries in children. A great deal of this preliminary research was organized by May Mellanby, who discovered an intimate relationship between vitamin D supplementation through a high vitamin D diet and a noteworthy reduction of caries occurrence in children.[33] Though former studies have implied that vitamin D is influential in preventing caries progression, there is still limited research in this area. Gaining further knowledge in this subject matter is essential, since early childhood caries remains a common and widespread problem amongst children worldwide.[40] This study aimed to determine if there was a relationship between levels of serum vitamin D, parathyroid hormone, and calcium in children with early childhood caries. An exploratory objective was to determine if vitamin D levels could be measured from the saliva of children.

Much of the current research found in this subject has acknowledged an intimate relationship between an acceptable vitamin D status and a decline in caries incidence. A study conducted by Schroth et al discerned that dental caries-free children were twice as likely to have ideal 25(OH)D measurements (≥75ng/mL) compared to those with severe-early childhood caries, who were three times a likely to exhibit deficient levels (<14ng/mL).[4] Our present study supports Schroth's previous findings, by illustrating that the vitamin D levels of children suffering from early childhood caries was statistically lower than our control population. When vitamin D levels are low, calcium is not fully absorbed and the body releases parathyroid hormone (PTH) to stimulate the mobilization of vitamin D from skeletal tissues.[4] Although not significant in our study, there was a trend for the PTH levels in the ECC children to be higher than the control group. In general, the calcium levels appeared to be comparable in both groups although we expected it to be reduced in the ECC group. It is possible that our particular sample population had an adequate intake of calcium obtained through their diet (dairy, milk) or that PTH was acting to maintain calcium homeostasis in our patients. However, since our study revealed no difference in the PTH or calcium levels of our groups, they cannot be recommended as measurements for caries risk in children. In addition, this current investigation also displayed a meaningful inverse correlation between the following variables: 1) vitamin D and the incidence of decayed, missing, and filled teeth (dmft) and 2) PTH and vitamin D levels. By confirming that an increased dmft is observed in conjunction with decreased vitamin D levels, we can further confirm a profound relationship between vitamin D and dental caries.

While vitamin D has been shown to alter an individual's caries risk, it is important to examine other factors that contribute to caries development since this disease is a complex, multifactorial process. It is highly recognized that financial barriers and employment status can heighten an individual's risk for caries, so it is important to consider these factors in addition to vitamin D status.[4] In general, although the analysis in this study demonstrated no significant differences between the two groups in regards to education level or employment, there were a greater percentage of caregivers in the caries group that either completed a lower level of education or were unemployed; additionally, more control caregivers completed advanced educational degrees (college or graduate school). A Canadian study confirmed the influence of education by proving that there was less decayed, missing, and filled teeth (dmft) in children whose parents completed a university degree.[41] Children whose parents only fulfilled an elementary school education and originated from lower income and socioeconomic backgrounds raised more children with active caries.[41] In addition, the chi-square analysis showed a statistical significant difference in income level, thus suggesting that the ECC children belonged to a lower socioeconomic group. A low socioeconomic status is a chief determinant for dental caries in children, with 80 percent of caries diagnosed in 20 percent of the population.[42] Frequently, unmet dental needs are more prevalent in lower income families due to the high cost of ECC treatment and the inability of these families to make dental care a priority.[40] Although a greater portion of our ECC children had Medicaid to assist in fulfilling treatment needs, Medicaid is still unpopular amongst dental providers and is poorly funded in a majority of states.[40] Likewise, a lower socioeconomic status oftentimes leads to decreased dental knowledge and increased caries, since the caretaker lacks the ability to comprehend the hazards of sugar intake. When the dietary habits of our groups were assessed, more ECC children and caregivers declared that they brushed their teeth less than the recommended frequency (at least twice a day). Many factors have an impact on the development of ECC, and they should be considered in addition to serum vitamin D levels so that preventive measures and programs can be implemented.

Lower socioeconomic groups often lack the means of acquiring nutritious food sources, so most of their diets comprise mainly of foods high in sugar and low protein.[42] One of the chief elements influencing the carious disease process is diet; in addition to impacting the progression of dental caries, diet has also been shown to increase the frequency of dental erosion and enamel defects such as enamel hypoplasia and fluorosis.[43] Guidelines endorsed by the American Academy of Pediatric Dentistry clearly state that this is caused by the prolonged contact of the consumed sugars and cariogenic bacteria on vulnerable teeth.[2] Our questionnaire exhibited that a statistically greater percentage of children suffering from early childhood caries had a snack before bedtime, ate snacks throughout the day, and consumed more beverages containing sugar. In addition to vitamin D levels, this is a critical element to take into consideration because a higher frequency of sugar intake, especially if ingested in-between meals (i.e., frequent snacking), has been implicated in children with high caries.

Vitamin D deficiency is a valuable indicator of dental caries, but it can also foretell a child's nutritional status. Children who are diagnosed with ECC may suffer from dental related pain, which can modify daily eating habits and cause these children to avoid the consumption of food, while simultaneously contributing to increased nutritional deficiencies within this population. In addition to poor nutrition, children with untreated dental caries endure undesirable consequences of the disease, including an increased risk for future caries, hospitalizations and emergency room visits, a rise in treatment costs, a possible delay in growth or development, school absences, limited activity due to discomfort, and an overall reduction in quality of life.[2] By avoiding the consumption of foods or by eating less nutritious foods, these children are often malnourished, which also predisposes them to vitamin D deficiency related dental caries.

Historically, a direct relationship has been found between vitamin D obtained from the sun, bone health, and dental caries. In addition to nutritional sources, direct contact with ultraviolet sunlight is considered to be the most ideal way to acquire sufficient vitamin D for use in the body.[23] Only 10 to 15 minutes of direct sunlight is needed in order to produce 10,000 to 20,000 IU of vitamin D. A study conducted in the 1930's observed that there was a marked decrease in the number of dental caries in 12-14 year old Caucasian males who resided in areas that received the greatest amount of sunshine.[35] Other studies have shown that children exposed to a larger dose of sunlight had less caries; these studies also found that even children artificially exposed to ultraviolet radiation, had fewer dental caries than children who were not exposed.[35] Some variables that modify ultraviolet radiation intensity and extent of exposure include a combination of: (1) atmospheric pollution due to dust, smoke, or fog, (2) temperature, (3) wind, (4) velocity, (5) cloud coverage, (6) total hours of sunshine, (7) light reflection from snow, (8) humidity, (9) latitude, and (10) altitude. Most of the above factors remained similar for our population since the most of the children lived near Virginia Commonwealth University or in surrounding areas. Though the responses in our questionnaire regarding sunshine exposure showed that only a slightly greater percentage of the children in the control group spent more time outdoors, this facet of vitamin D should still be monitored. It could be that there were inaccuracies when this data was recorded and caregivers were merely estimating or making conjectures about their child's actual sun exposure. Moreover, individual responses may vary depending on what season or time of year the survey was completed. Several reports of a seasonal incidence of caries have been recorded, with the greatest amount of new caries being detected during the winter.[44] Due to the colder temperatures in the winter, children may be compelled to remain indoors more often and subsequently, receive less vitamin D from ultraviolet radiation; also, the diverse forms of available winter clothing required for lower temperatures may affect the degree of sunlight exposure on the skin.[35] Thus, one would expect a dramatic escalation in sun exposure during the summer or spring months in comparison to the colder months. Supplementary exposure to vitamin D from the sunlight during the warmer months elucidates why there is a decline in caries occurrence during these periods.[44]

There is also widespread evidence of racial and ethnic disparities in the distribution of dental caries between various groups.[45] The prevalence of early childhood caries has been shown to be prodigiously elevated amongst the low income and minority populations, especially in the Native American, Hispanic, and African American groups residing in the U.S.[40] The analysis in this study indicated a statistically significant difference in regards to race amongst the groups, with a preponderance of the ECC children self-identifying as African-American and more control children being Caucasian. Current evidence indicates that because a higher percentage of African Americans have suboptimal levels of vitamin D, they have an augmented predisposition for several medical conditions (cancer, diabetes, osteoporosis) and dental caries. It is also hypothesized that individuals with darker pigmentation have diminished vitamin D production in their skin, resulting in lower 25(OH)D levels.[46] In order to attain equivalent levels of serum vitamin D, it is necessary for those with the characteristic dark pigmentation to spend 5 to 10 times more time outdoors exposed to sunlight, when compared to infants and children with lighter pigmentation.[23] Since previous research suggests that the African American population in our study would need to spend more time outdoors in order to produce an equivalent amount of vitamin D, this could also explain why their overall serum vitamin D levels were lower in this group. Certain articles also claim that African Americans have a lower dietary vitamin D intake due to an increased incidence of lactose intolerance in this group, which causes a lower intake of vitamin D containing milk products and fortified breakfast cereals.[23] The escalated occurrence of poor vitamin D levels in the African American population makes them more susceptible to developing vitamin D related dental caries and should also encourage providers to make proper recommendations when this is discovered, so that potential health benefits can be obtained.

It is important for health professionals to be cognizant of the vitamin D levels of their patients since nutritional deficiencies in this hormone has the potential to adversely impact the well-being of these children. It is estimated that between 12-24 percent of infants, children, and adolescents have a deficiency in this vitamin although there is an array of food sources that contain it.[23] Practitioners should make themselves familiar with this issue, so that they are ready to inform caregivers about vitamin D nutrition if needed. The American Academy of Pediatrics highly recommends that all infants, children, and adolescents consume a minimum of 400 IU of vitamin D daily.[23] When being used as a supplement, vitamin D_3 (cholecalciferol) is the desired form of vitamin D. It is also recommended that children who are either at risk or are currently diagnosed with vitamin D deficiency have their 25-hydroxyvitamin D levels monitored every 3 months and their PTH and bone mineral status every 6 months. Medical and dental professionals should comprehensively explain to these caregivers that by modifying their child's vitamin D status early in life, they can also prevent osteoporosis in adulthood by modifying bone mass and benefits that incorporate areas beyond the skeletal system (i.e., reduction of dental caries) can also be seen.

An exploratory goal of our study was to determine if vitamin D levels could be analyzed in saliva. Aside from performing numerous functions in the oral cavity (maintaining oral health, acid clearance from dental plaque, contributes to the re-mineralization of early carious lesions, etc.), saliva is a valuable diagnostic tool that can be used to assess an individual's health and well-being.[37,47,48] Since whole saliva provides clinicians an uncomplicated, non-invasive manner in which to gather repeated data sampling, it has drawn recent interest as an innovative approach to measure compounds and hormones for the diagnosis of a number of medical conditions.[38]

Unfortunately, the saliva assay that was developed for this study to measure salivary vitamin D was ineffective when tested in children. The principal weakness in our study was that there was no reliable method in which to collect an adequate amount of saliva from the sample patients. Age and anxiety were factors contributing to the inability of children to expectorate. Fasting prior to the procedure may have contributed to the struggles in obtaining an adequate sample. Although our saliva assay was incapable of measuring salivary vitamin D in children, previous studies were able to detect vitamin D in saliva. For example, the study by Higashi et al was effective in measuring $25(OH)D_3$ accurately in un-stimulated saliva.[38] It is imperative that an accepted method for measuring vitamin D in saliva be established, since it has the capacity to become a more accepted and cost-effective technique in diagnosing disease in children.

This study had several limitations. Caregivers were requested to complete the questionnaire the morning before their child's surgery, so it is a possibility that they did not have ample time to read through the questionnaire which caused caregivers to misinterpret and inaccurately answer questions, or leave some questions unanswered. Some caregivers may have hurried through the survey because they were probably more focused on nurturing their children prior to the commencement of their treatment or had anxiety and concerns about their child undergoing general anesthesia. There were several incomplete questionnaires that were collected during the course of this study and two uncollected questionnaires, which may be attributed to an increase in the uneasiness amongst the caregivers. Numerous attempts to contact the caregivers of the uncollected questionnaires were made throughout the course of this study. Secondly, there were a number of discrepancies with the results of the questionnaire because it was modified during the study and not all caregivers completed the same version of it. The original questionnaire was revised during the study to make the responses more specific and easier for the caregiver to interpret. The questions that were modified discussed the child's quality of sun exposure, brushing frequency, how often the child went to bed with a bottle, snacking frequency before bedtime, and caregiver brushing habits. One question regarding the child's use of fluoridated toothpaste was also added into the new questionnaire. There was also a difference in the final number of controls and ECC patients (20 and 38, respectively).The addition of more controls may alter the results of the study, leading to greater statistical power and permitting more certainty in our results.

The majority of the controls were obtained from patients that were scheduled for an Ear, Nose, and Throat (ENT) surgical procedure with the VCU Otolaryngology department. Collecting serum samples from healthy patients of the VCU Otolaryngology department was less difficult, since the child was undergoing general anesthesia and already needed an intravenous line for treatment. There is a possibility that this introduced a bias and that this sample may not be a true representation of other populations. Identifying caries free controls and ECC children with similar characteristics (race, socioeconomic status, and education) was also difficult to control. Another limitation in this study was the visual clinical exam in control patients and the lack of radiographs; there is a possibility that some of the control children had undiagnosed caries.

Due to inspiring discoveries and endless possibilities in this field of research, future studies should continue to expand on the significance of this hormone in our lives. An area that needs additional research is determining the prenatal association with vitamin D and the development of dental caries and dental anomalies. Presently, there are studies that suggest an association between a mother's health both prior to and during pregnancy, breastfeeding, and prenatal supplementation. Schroth et al observed that defects in enamel are associated with difficulties encountered during the prenatal and early postnatal periods.[49] Specifically, an in utero deficiency in vitamin D results in a metabolic insult to ameloblasts and consequently, results in the development in enamel hypoplasia. Vitamin D is also integral in maintaining both calcium and phosphorous homeostasis, so that the calcification of hard tissues can occur; hence, maternal deficiency in vitamin D during pregnancy may disrupt proper tooth calcification, making the child more susceptible to enamel hypoplasia and ultimately, dental caries. To examine the relationship between prenatal vitamin D and dental caries in an infant's first year of life, Schroth et al performed a prospective cohort study administering a prenatal questionnaire and collected a serum sample from expectant mothers.[5] When the infant reached his or her first year of life, dental examinations were performed to assess for enamel hypoplasia, cavitated and white spot lesions. The researchers of this study discovered that mothers of children diagnosed with early childhood caries had suboptimal vitamin D levels that were statistically lower compared to children who were caries-free. Since this was the first time that prenatal vitamin D levels were shown to influence the development of early childhood caries, supplementary studies are indispensable in expanding and diversifying our awareness in this matter. Prospective studies may also be interested in a more biological aspect of measuring vitamin D such as extracting vitamin D measurements from the *funiculus umbilicalis* (umbilical cord) to assess prenatal interchange of vitamin D between unborn child and mother. Because experiments have found that a mother's vitamin D status can significantly reduce the rate of enamel defects and caries in their children, it is important to completely comprehend this connection so that proper recommendations for vitamin D supplementation can be given to the mother during her prenatal care.

Since previous research has implicated low vitamin D levels to several health risks, upcoming studies should also inquire how vitamin D parameters are influenced by the effects of asthma, obesity, preterm births, or skin pigmentation. Additionally, this current study should be repeated using ECC and control patients that share similar characteristics (race, socioeconomic status, caregiver education), to eliminate possible risk factors that may influence the patient's caries risk. The discovery of the increasing importance of vitamin D to our health substantiates the need for further studies in this area.

Though there is convincing evidence linking vitamin D and caries, much of the past research has been left unnoticed.[5] Our current findings verify previous studies by Schroth et al and Mellanby, which further validates the significance of vitamin D in relation to dental caries and should stimulate supplementary research in this area.[5,33] In addition to dental caries, vitamin D deficiency has been implicated in several adverse health conditions such as: bone disorders, respiratory diseases (asthma), cardio-metabolic diseases, immune function, insulin resistance, obesity, preterm births, cancers .[8,20,49,50] Considering the high prevalence of vitamin D insufficiency or deficiency in children and adolescents, it would be advantageous for healthcare providers (medical personnel and dentists) to be knowledgeable about its importance since it is fundamental in achieving optimal health and well-being for our children.

Conclusion

In summary, our study revealed the following:

- The difference in the vitamin D levels of the control group and ECC group were statistically significant. The ECC group demonstrated deficient levels of vitamin D, whereas the control group displayed sufficient levels.
- There was also a significant, inverse correlation between the following serum values: vitamin D and PTH and most importantly, vitamin D and dmft.
- Saliva offers a non-invasive and innovative way to measure vitamin D; future studies should continue to explore this method although our assay was unable to assess this parameter in children.

The results of this study suggest a strong relationship between vitamin D and children with early childhood caries. It is recommended that healthcare providers (medical doctors and dentists) become familiar with their patient's vitamin D levels when deciding on treatment options for maintaining overall health and well-being. Ultimately, making proper suggestions for vitamin D supplementation may be beneficial in reducing a child's caries risk and will eventually diminish the need and costs of dental treatment.

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Tables

Table 1. Classification of Vitamin D, PTH and Calcium Levels

Vitamin D (ng/ml)	Sufficient	>30-100
	Insufficient	20-30
	Deficient	<20
PTH (pg/ml)	High	>65
	Normal	12-65
	Low	<12
Calcium (mg/dL)	High	>10.9
	Normal	9.1-10.9
	Low	<9.1

Table 2. Demographic Characteristics of the Participants

Characteristic	Count: Control	Count: ECC	p-value
Race[1]			
Caucasian	15	14	*0.0119
African American	5	23	*0.0135
Hispanic	1	2	1.0000
Asian	1	0	0.3448
American Indian or Alaskan Native	0	2	0.5402
Native Hawiian or Pacific Islander	0	1	1.0000
Education level			0.0830
high school or less	6	22	
college	10	10	
graduate school beyond college	4	4	
Caregiver employed			0.7264
yes	16	26	
no	3	8	
Household Income level			*0.0003
<30,000	4	18	
30,000-49,999	0	5	
>50,000	15	6	
Age (months)			*0.0028
Mean	37.5	50.8	
SD	18.15	13.90	

1 Since race/ethnicity are "check all that apply", the counts do not add up to the number of patients in each group

p-value < 0.05

Table 3. Medical History and Sun Exposure of the Participants

	Count		Percent (%)	
Characteristic	Control	ECC	Control	ECC
Medical Problems				
ADHD	1	1	5	3
Asthma	2	5	10	13
ENT Problems	9	1	43	3
Heart Murmur	1		5	
Premature	4	7	19	18
Seasonal Allergies		3		8
Seizures	1	3	5	8
Healthy	3	18	14	47
Sun Exposure	Control	ECC	Control	ECC
Daily	12	22	86	79
Monthly		1		4
Weekly		3		11
None	2	2	14	7

Table 4: Dietary Habits of the Participants

Behavior	Count: Control	Count: ECC	Percent (%): Control	Percent (%): ECC	p-value
Bottle/ sippy cup					0.5699
yes	8	12	42	34	
no	11	23	58	66	
Bed w/bottle					0.1710
never	18	28	95	78	
daily	0	5	0	14	
weekly	1	3	5	8	
Snacks before bedtime					*0.0120
never	18	14	90	41	
daily	2	19	10	56	
monthly	0	1	0	3	
Snacks throughout the day					*0.0063
never	2	0	10	0	
daily	12	31	60	86	
weekly	6	5	30	14	
Sugary drinks					*0.0151
never	4	0	21	0	
daily	12	29	63	81	
weekly	2	6	11	17	
monthly	1	1	5	3	
Tap water					0.2986
never	3	8	15	22	
daily	16	22	80	61	
weekly	1	6	5	17	

p-value < 0.05

Table 5. Dental History of the Participants

Characteristic	Count Control	Count ECC
Child's dental care		
Fluoride in water		
yes	13	13
no	5	8
don't know	1	6
Fluoride supplement		
yes	3	6
no	17	27
don't know	0	2
Fluoride treatment		
yes	7	16
no	12	15
don't know	1	4
Brushing frequency		
daily	18	30
weekly	0	4
never	2	2
Flossing frequency		
daily	3	10
weekly	5	4
monthly	0	3
never	10	19

Characteristic	Count Control	Count ECC
Caregiver's dental care		
Dental visits		
yearly	12	32
never	8	0
only when in pain	0	3
Difficulty getting to dentist		
yes	0	9
no	20	27
Caregiver caries		
yes	14	24
no	6	11
Caregiver have natural teeth		
yes	20	36
Caregiver brushing		
daily	20	35
never	0	1
Medicaid		
yes	9	27
no	11	7
Caregiver insurance		
yes	16	18
no	4	18
Public assistance		
yes	4	11
no	16	24

Table 6. Serum Vitamin D, PTH and Calcium of the Participants

	Group	n	Mean	SD	SE	p-value
Vitamin D (ng/ml)	Control	20	32.4	8.57	1.92	
	ECC	38	26.6	8.27	1.34	
	Difference		-5.8		2.31	*0.0150
PTH (pg/ml)	Control	20	47.4	21.34	4.77	
	ECC	38	57.5	28.84	4.68	
	Difference		10.1		7.33	0.1724
Calcium (mg/dL)	Control	20	9.6	0.38	0.08	
	ECC	38	9.6	0.28	0.05	
	Difference		0.0		0.09	0.6673

p-value < 0.05

Table 7. Vitamin D, PTH and Calcium Levels of the Participants

	Group	Count			p-value
		D	I	S	
Vitamin D	Control	0	9	11	
	ECC	7	20	11	*0.0455
		L	N	H	
PTH	Control	0	17	3	
	ECC	1	23	14	0.1146
		L	N	H	
Calcium	Control	1	19	0	
	ECC	1	36	1	0.5920

p-value < 0.05

Table 8. Correlations between Measurements

Pairwise Correlations

Variable	by Variable	Correlation	Count	Lower 95%	Upper 95%	Signif Prob
PTH	Vit D level	-0.38	58.0000	-0.5836	-0.1384	*0.0030
Calcium	Vit D level	0.00	58.0000	-0.2542	0.2624	0.9737
Calcium	PTH	-0.11	58.0000	-0.3579	0.1529	0.4120
dmft	Vit D level	-0.33	58.0000	-0.5422	-0.0785	*0.0114
dmft	PTH	0.16	58.0000	-0.1053	0.3994	0.2383
dmft	Calcium	0.01	58.0000	-0.2518	0.2648	0.9587

p-value < 0.05

Table 9. Total Serum Levels for Both Groups

Column	N	DF	Mean	Std Dev	Sum	Minimum	Maximum
Vit D level	58	57.00	28.6328	8.7538	1660.7	9.6	47.3
PTH	58	57.00	54.0431	26.7477	3134.5	10.2	120.2
Calcium	58	57.00	9.6000	0.3173	556.8	8.6	10.5
dmft	58	57.00	7.8276	6.3692	454	0	20

Figures

Figure 1. Vitamin D Synthesis and Metabolism

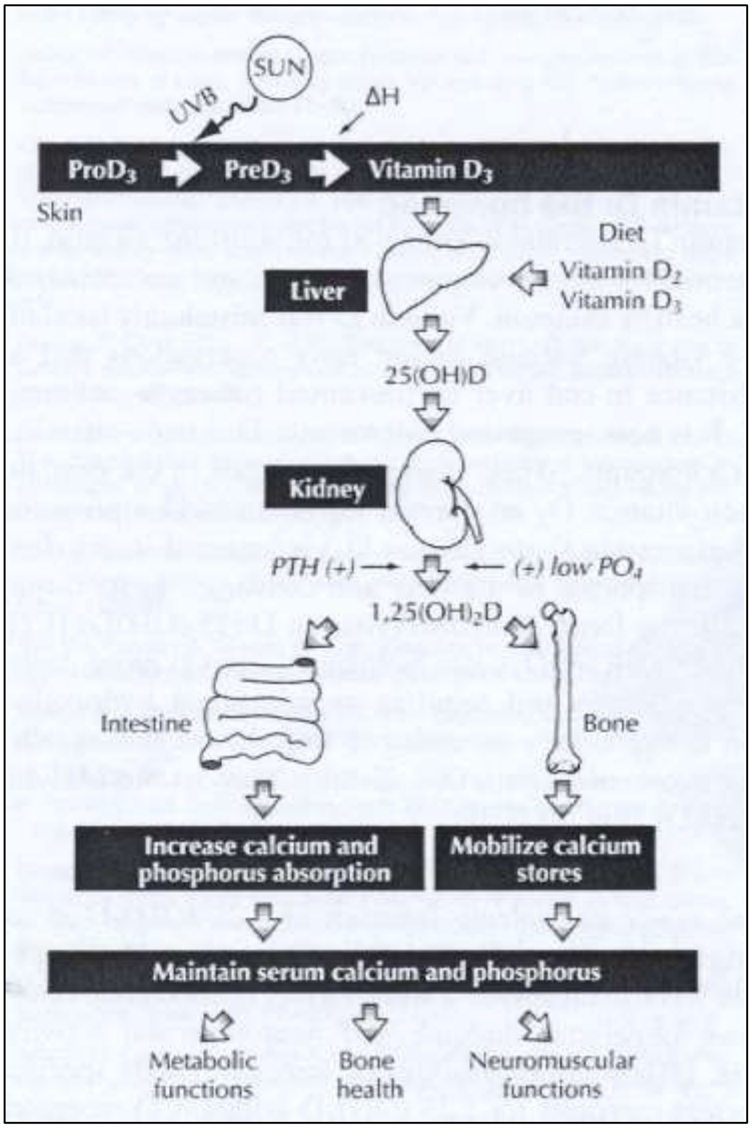


Figure 2. Vitamin D Serum Levels

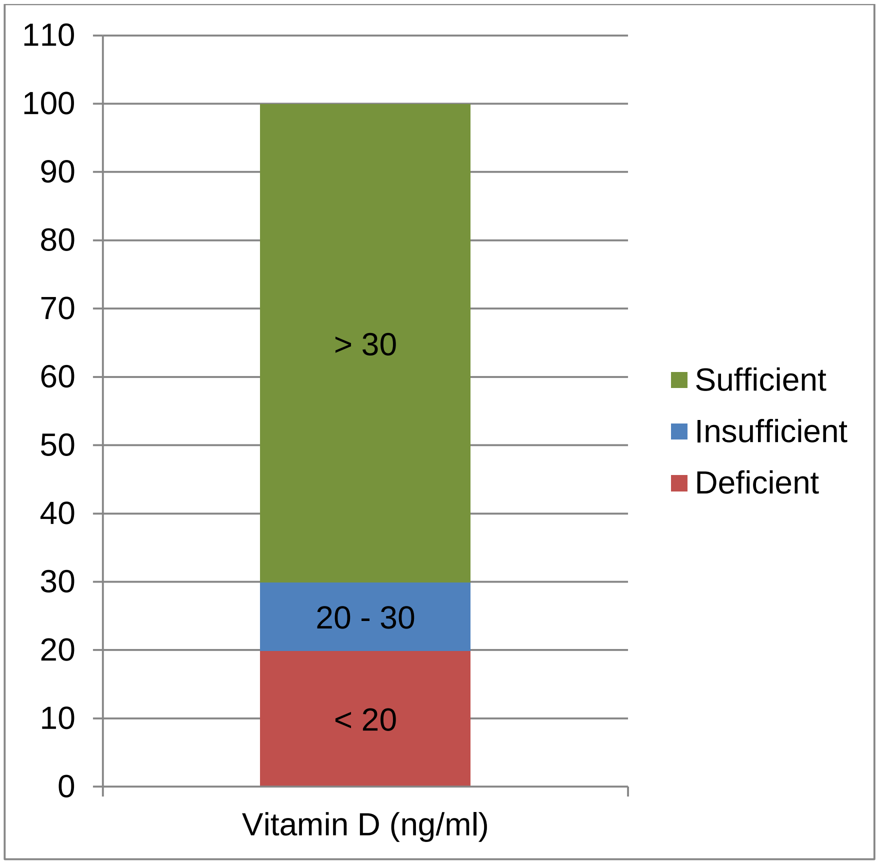


Figure 3. PTH and Calcium Serum Levels

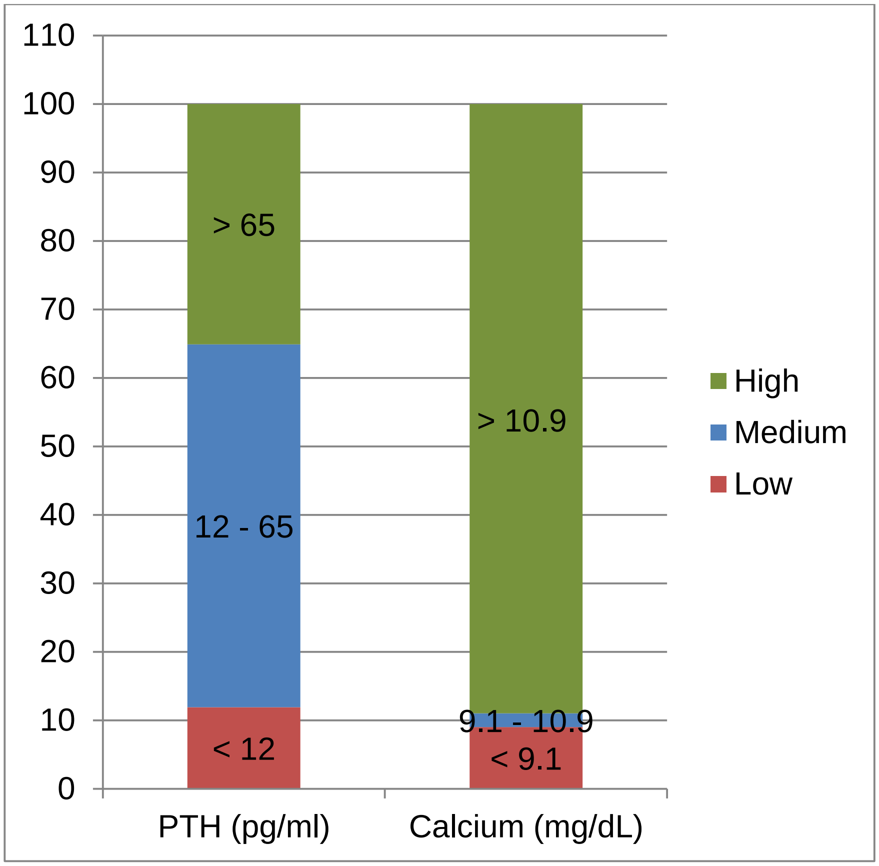


Figure 4. Mean Serum Vitamin D Levels

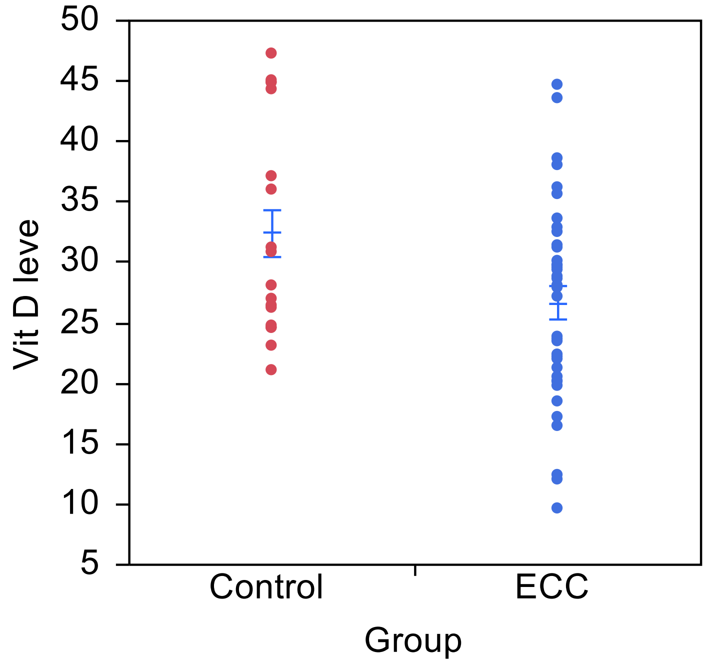


Figure 5. Comparison of Vitamin D Levels of the Participants

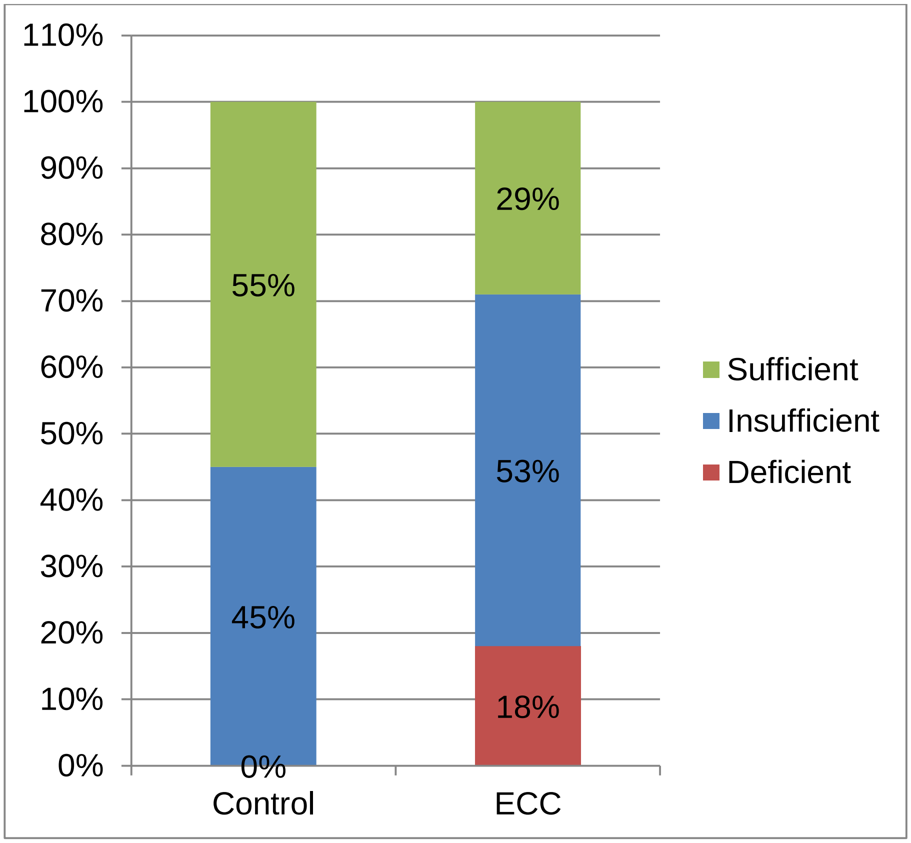
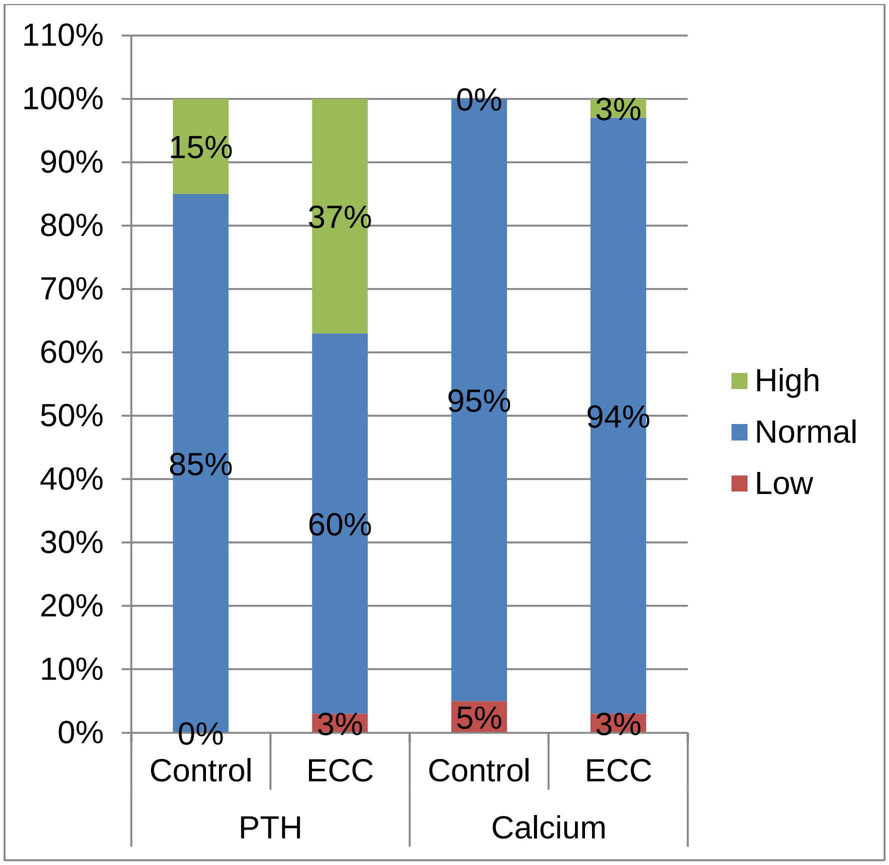


Figure 6. Comparison of PTH and Calcium Levels of the Participants



Appendix A

Questionnaire for the primary caregiver: Original Questionnaire

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	☐ Three or more times a

as raisins, candy, cookies, cakes, or cereal between meals?	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.

Questionnaire for the primary caregiver: Revised Questionnaire

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 does your child play outside during the day?	None At least 1hr/day 1-2hrs/day More than 2 hrs/day

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. Is there fluoride in your drinking water at home?	Yes No Well Water
4. 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)
5. How often does an adult brush your child's teeth with toothpaste?	☐ Once a day ☐ Twice a day ☐ Three times a day ☐Never
6. Does your toothpaste have fluoride?	Yes No Don't Know
7. How often are your child's teeth flossed?	Daily Weekly Monthly Never

Next we ask about your child's eating habits	(Select one)
8. Does your child usually (throughout the day) drink from a bottle or sippy cup?	Yes No
9. How often does your child go to sleep while nursing or while drinking something other than water from a bottle/sippy cup?	☐ Three or more times a day ☐ One or two times a day ☐ Weekly ☐ Monthly ☐Never
10. After nighttime brushing, does your child eat or drink anything other than water before bed?	Yes No
11. 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
12. 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
13. 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)**
14. How often do you take your child to the dentist?	☐ Never ☐ Only when in pain ☐ Yearly ☐ Twice a year
15. Is it very difficult to get your child to the doctor or dentist?	Yes No
16. Is your child's care covered by Medicaid or State Insurance?	Yes No Don't Know
17. 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
18. Does your child participate in public assistance programs (ex: WIC, Healthy Start, etc.)?	Yes No Don't Know

Questions about your (the caregiver's) teeth.

Now we will ask about your teeth and your tooth care	**(Select one)**
19. Do you have cavities or fillings or have had teeth pulled in the last 2 years?	Yes No
20. Do you have any of your own natural teeth	Yes No
21. How often do you brush your teeth?	☐ Once a day ☐ Twice a day ☐ Three times a day ☐Never
22. Do you have dental insurance?	Yes No
23. How often do you get dental checkups?	☐ Never ☐ Only when in pain ☐ Yearly ☐ Twice a year

Now tell us a little bit about you…	
24. What is the highest level of education that you completed?	☐ Elementary and Middle School ☐ High School ☐ College ☐ Graduate school beyond college
25. Is an adult in the child's household employed?	Yes No
26. 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

Vanessa Ong Hofileña was born on November 8, 1982, in Olongapo City, Philippines and is an American citizen. She graduated from Indian River High School, in Chesapeake, Virginia in 2000. She received a Bachelor of Arts in Biology and Psychology and a minor in Spanish from The University of Virginia, Charlottesville, Virginia in 2004. She also received a Doctorate of Dental Surgery from Virginia Commonwealth University School of Dentistry in 2008.

After graduation, she joined the United States Navy and completed an Advanced Education in General Dentistry with the military at Sewell's Point Dental Clinic in Norfolk, Virginia in 2009. After completing her certificate, she was stationed at Cherry Point, North Carolina with the 12th Dental Battalion from 2009-2011. While at Cherry Point, Vanessa deployed to Haiti in 2010 with the 22nd Marine Expeditionary Unit aboard the USS Bataan to provide earthquake relief and emergency treatment for the people of Haiti. After being stationed at Cherry Point, she was stationed at the Washington Navy Yard in Washington D.C. from 2011-2013. Vanessa 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.