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Comparing tooth enamel disturbances in a pediatric population that had received prior chemotherapy treatment to age-matched controls from the Virginia Commonwealth University Pediatric Dentistry Clinic.

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Comparing tooth enamel disturbances in a pediatric population that had received prior chemotherapy treatment to age-matched controls from the Virginia Commonwealth University Pediatric Dentistry Clinic

A thesis submitted in partial fulfillment of the requirements for the degree of Masters of Science

in Dentistry at Virginia Commonwealth University.

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Abstract

COMPARING TOOTH ENAMEL DISTURBANCES IN A PEDIATRIC POPULATION THAT HAD RECEIVED PRIOR CHEMOTHERAPY TREATMENT TO AGE-MATCHED CONTROLS FROM THE VIRGINIA COMMONWEALTH UNIVERSITY PEDIATRIC DENTISTRY CLINIC.

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

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Purpose: The purpose of this study was to test whether children who have undergone cancer chemotherapy have a higher prevalence of enamel abnormalities of the second mandibular premolars than age matched controls.

Methods: This study was a case-control design where the case group involved 26 subjects that had received chemotherapy treatment between the ages of 2 to 6 and at the time of the study were between the ages of 9 to 18. The control group consisted of 26 subjects matched for age and sex to the case group that had not received chemotherapy.

The second mandibular premolars were assessed based on the types of defects, their number and location according to a modified DDE index. A secondary examiner, blinded to the

results of the primary, analyzed photographs taken at examination and provided their own assessment in order to calibrate results.

Result: Nominally there were more normal surfaces in the case group than in the control group (81% vs 70%) and fewer hypoplasias in the case group (5% vs 13%). There was no statistical difference between the buccal and occlusal surfaces. For the buccal surfaces, the cases were nominally lower but not statistically significant (P=0.0680) and there is no evidence for a case-control difference on the lingual surfaces (P>0.9).

Conclusions: In this study developmental defects of the enamel organ were not observed to be statistically different between the case and control groups, although previous studies have shown otherwise.

Introduction

Today the number of children surviving cancer is increasing exponentially. With that said, clinicians are becoming more aware of the possible long term side effects and sequelae of cytotoxic chemotherapy. [1] [2] Damage to the dentition is one such concern; since the developing teeth are sensitive to systemic disturbances. These are likely to produce a physiological function disturbance in the dentition that may cause ameloblasts to form enamel abnormally. [1]

Chemotherapy is selectively toxic to active proliferating cells by interfering with DNA synthesis and replication, RNA transcription and cytoplasmic transport mechanisms. Chemotherapy interferes with the cell cycle and with intracellular metabolism, and therefore lead to dental abnormalities. [3] With respect to the effects of antineoplastic therapy in dental development it should be noted that children that received chemotherapy during the early years of their lives, a period associated with the development of teeth, presented with disturbances in the formation of enamel. [4]

The immediate effects of chemotherapy and radiotherapy in soft and hard tissues are well documented, but less is known about the effects of chemotherapy itself in developing dental tissues. Animal studies have shown that chemotherapeutic agents induce qualitative and quantitative changes in dental tissues and odontogenesis, as well as inhibition of eruption. The extent of these abnormalities depends on factors such as the type of chemotherapeutic agent used, half-life of the agent and the number of cells in susceptible phases of the cell cycle. [5]

Enamel is the hardest substance in the human body and due to its high mineral content and organized structure, enamel has exceptional functional properties. We know that mature

enamel is mainly composed of carbonated hydroxyapatite which is made up of long and narrow crystals, packed into parallel arrays called enamel rods. The extracellular enamel matrix proteins secreted by the ameloblasts during amelogenesis suggest that this orchestrated extracellular process regulates nucleation, growth and organization of forming mineral crystals. [6] However, the process is not completely understood because the organic matrix does not persist when enamel is mineralized. [6 7]

Dental enamel is elaborated by ameloblasts. As it is being formed this structure is highly hydrated with organic matrix and a low concentration of inorganic-apatite. Prior to the eruption of the tooth, this extracellular organic matrix undergoes numerous changes resulting in an increase of the inorganic phase and withdrawal of the organic structure and water. [6 7]

This meticulous process is what we call “enamel maturation”, mineralization or calcification where the organic structure of the enamel is removed during early stages of mineralization and replaced by an increasing amount of mineral content. Subsequently, water decreases, resulting in a further increase of enamel density which is associated with progressive hardening of the enamel structure from very soft to very hard. [8]

More recent studies have suggested that human enamel is formed in two distinct stages. In the first “secretory stage”, the long thin ribbon of enamel is formed immediately as the ameloblasts lay down enamel matrix proteins. [9] In this stage, the mineral phase of secretory enamel is approximately 10 to 20% of the volume, with the remaining portion occupied by matrix protein and water. When the thickness of the enamel and elongation of the crystals are established we pass into the second stage known as the “maturation stage”. [6] This coincides with the almost complete removal of enamel proteins by proteases and a rapid increase in mineral concentration suggesting that this second stage initiates upon completion of the enamel matrix.

The mineralization advances from the earliest formed matrix at the cuspal portion of the enamel dentin junction peripherally and cervically in a pattern approximating the incremental deposition of enamel matrix. [7]

Up to this date, evidence suggests that this formation process is a protein-protein and protein-mineral interaction, having amelogenin as the predominant enamel matrix protein. These proteins are highly hydrophobic, rich in proline, glutamine, leucine and histidine. Although amelogenin is processed by proteinases shortly after its secretion, the intact full molecule is found exclusively in the region of the newly formed enamel. [9]

Enamelin and ameloblastin may also have relevant importance in the enamel formation. Enamelin is a hydrophilic and acidic protein, rich in glycine, aspartic acid and serine. Like amelogenin, enamelin undergoes gradual enzymatic degradation extracellularly, suggesting that various degradation products of enamelin have different roles in amelogenesis. [6] The latter, was suggested to play a role in crystal growth based on its location relative to the Tomes process which is the secretory end of ameloblast and the site of crystal growth initiation. It has also been proposed that ameloblastin may also be a cell adhesion molecule that facilitates the attachment of the ameloblasts to the enamel matrix. [6] This factor may be essential for the maintenance of the ameloblasts in their differentiated state, which is ultimately required for proper enamel deposition. [10]

During enamel formation, ameloblasts are susceptible to local trauma, hereditary conditions and systemic metabolic disturbances. These disturbances may be reflected in the fully formed enamel as hypoplasia, hypocalcification or accentuated incremental lines. All consequential effects depend on the intensity, duration of the etiologic factor and when the derangement occurs during crown formation. [11] Hypoplasia which is caused by local factors or

trauma will affect only one tooth and sometimes the adjacent tooth, whereas hereditary defects will begin at birth and affect the entire tooth crown. [8] Enamel hypoplasia is caused by a disturbance in the ameloblasts during tooth formation expressed by alterations of ameloblastic reproduction, secretory function, membrane permeability and calcium exchange across the cell membrane. Hence, the tooth enamel often acts as a repository of information on the systemic damage received during development. [12] These alterations are significantly more common in children surviving cancer. Children who had been treated for Acute Lymphoblastic Leukemia seem to be more severely affected and this may be reflected in the longer duration of therapy leading to a greater risk of affecting ameloblasts. [3]

Among the drugs used for cancer treatment Vincristine (VCR) and Vinblastine (VBL) are widely used as anti-tumor agents. Biochemical and ultra-structural studies have shown that these drugs interact with tubulin molecules. [13] These are complex protein subunits of microtubules, and in some cell types the alkaloids cause them to aggregate into a large cytoplasmatic crystalloid structure. As a result, cellular function dependent on the microtubules suffers and cells in mitosis are irreversibly stopped in metaphase which can result in an abnormal appearance of the enamel. [14] Some of the changes and effects are a combination of the interaction with the microtubules, inhibition of collagen and general protein, increased autophagic activity as well as necrotic changes. [13]

Previous studies on effects of VCR on dentinogenesis indicated that this drug has immediate, varied and dose-dependent effects on several stages in the process. In some cases, VCR produced an accumulation of numerous abnormal metaphases of pre-odontoblasts in the apical part of the pulp near the terminal odontogenic epithelium. Other cases showed that metaphases were not observed among fully developed odontoblasts and therefore they lost their capacity to

proliferate. [13] The results showed that VCR effects differed across regions of the tooth. The number and disposition of odontoblasts was reduced in all areas of tooth sections, especially in the pulp horn. In the central part of coronal and radicular pulp tissue blood vessels were dilated and filled with blood cells. The histologic appearance of newly formed dentine was irregular. [15]

Doxorubicin is a drug widely used as an antineoplastic agent that damages DNA. This drug is used to treat many cancers in children. Due to recent advances in cancer treatment, there is a growing population of young patients with long-term side-effects from chemotherapy including abnormalities of the dentition, which may occur in teeth undergoing odontogenesis during treatment. [16] The effects of continuous exposure to therapeutic concentrations of doxorubicin are undetermined. All dilutions of doxorubicin significantly inhibited fibroblast cell proliferation. The dental pulp itself is a loose connective tissue containing various subpopulations of cells, including fibroblasts and undifferentiated cells capable of transformation into mineralized tissue. In addition, doxorubicin has been reported to produce dentinal hypoplasia in humans. [16]

Considering that tooth enamel can be affected by adverse biological events, the changes in structure can provide clues to the time and nature of these events. Therefore, enamel defects can then be studied as a marker of many adverse biological events that occurred during their developmental phase, and that may have applications in clinical and epidemiological investigations. [17] Improving knowledge and understanding of these effects would make it possible to improve clinical management, educate parents that normal dental structures may be affected and offer a list of solutions to mitigate any impact. [2]

Thus, the aim of the study was to establish a positive correlation between chemotherapy and enamel abnormalities of the second mandibular premolars in the pediatric population that

received chemotherapy treatment from two to four years of age. The reason we focused on the second mandibular premolars is because the enamel for the second mandibular premolars forms between the ages of two to six – which is when the case subject population had received chemotherapy treatment. If the findings are positive then the results will make it possible to:

- Provide proof that in the Pediatric Dental community, there is a positive correlation between chemotherapy and alterations in the enamel formation. This will help determine the best approaches to help patients who may develop dental issues due to defective tooth enamel.
- Educate parents of children that will be receiving chemotherapy that normal dental structures may be affected and offer a list of solutions to resolve such issues.
- Educate parents of children that have received chemotherapy in the past that normal dental structures may have been affected by the procedure while also offering a list of solutions to mitigate any impact.

Methods

Study Design

This study was approved by the VCU Institutional Review Board (IRB). Approval #: HM15036. This study utilized a case-control design where the case group involved subjects that had received chemotherapy treatment between the ages of 2 to 6. At the time of the study, subjects were between the ages of 9 to 18, which is when the mandibular second premolars should be fully erupted.

The case group was accessed from the Long-term Survivor Clinic located at St. Mary's Hospital, which is part of the Pediatric Hematology and Oncology division of the Virginia Commonwealth University Health Systems. The subjects were addressed during their follow-up appointments with a script presenting the purpose of the study.

The control group consisted of up to 69 subjects matched for age and sex to the case group and were accessed randomly through the Pediatric Dental Clinic at Virginia Commonwealth University, School of Dentistry (VCU). They were addressed during their six months periodical examinations with a script presenting the purpose of the study.

The following exclusion criteria applied to all subjects:

- Previous trauma in the premolar area
- Diagnosed with hereditary conditions or syndromes
- Received endodontic treatment in the second mandibular premolar
- Received or currently in orthodontic treatment
- Teeth that are partially erupted
- Not living in a fluoridated water community

Data Collection

Informed consent was obtained from parents of those participating in the study. Parents completed a questionnaire about the subject's health history. Subjects were examined in a semi supine position. Intraoral examination with a disposable dental mirror was performed on all surfaces of the second mandibular premolar. Teeth were examined without previous prophylaxis, but debris was removed from the individual sites, where visibility was compromised, by using 4x4 gauze. This assessment was based on the types of defects, their number and location. The type and the location of the developmental defects of the enamel were classified according to the modified DDE index introduced by Clarkson and O' Mullane. [18]

After examination, the second mandibular premolar was photographed using a Canon 2Ti camera with a 90mm Tamron portrait lens at a reduction of 1:32 with a Dine Corp. ring flash or point flash. The buccal, lingual and occlusal surfaces of #20 and #29 were photographed directly. Photos were taken in order for the secondary examiner to analyze enamel defects and provide their own numerical value according to the same modified DDE index used by the primary examiner. This was done in order to calibrate results.

Classification of Developmental defects

For the purpose of the study, the type and location of the developmental defects of enamel were classified according to the modified DDE index (Table 1). [18]

Statistical Methods

To compare the ages in the cases and controls, a test of equivalence was used. The cases and controls were compared using a likelihood-ratio chi-square test or a t-test, as appropriate. The

cancer, leukemia, and control patients are compared using Wilcoxon's rank sum test. The test of the specific aim was accomplished using two analyses: Considering DDE as a binary outcome, a repeated-measures logistic regression included the following factors in the model: Case versus control, sex, age, tooth, and surface. Considering DDE as a numerically scored value (0 to 3), a repeated-measures mixed-model ANOVA included the same factors in the model plus it considered that the group differences may vary by surface. All analyses were performed using SAS software (SAS version 9.3, JMP version 10.0, SAS Institute Inc., Cary NC).

Results

First, the results of screening and patient recruitment are described. Then the demographic, medical and dental characteristics of the cases and controls are compared. The primary analyses describe the differences in developmental defects of the enamel in the main section. And finally, the cases are divided into those with cancer and those with leukemia and these are compared to controls.

Patient recruitment

The clinic had on record 456 children that were between the ages of 9 to 18. Of these, 95 received chemotherapy between the ages of 2 to 6. Of the 95, 19 have died, 6 have moved away and are no longer followed by the clinic, and 1 had relapsed and is back on treatment. This left 69 prospective cases between the ages of 9 to 18 that received treatment between the ages of 2 to 6. Of the 69 potential subject cases, 26 were eventually recruited for the study. 43 subjects were excluded due to one or more of the following reasons:

- Diagnosed with hereditary conditions or syndromes
- Received endodontic treatment in the second mandibular premolar
- Received or currently in orthodontic treatment
- Teeth that were partially erupted
- Scheduling constraints – note: this was the primary reason for exclusion, and the primary source of potential bias

The 26 cases were diagnosed most commonly as ALL (42%, n=11) and next most commonly with Wilm's tumor (19%, n=5). Additionally there were 6 cases of ALL, and one

each of the following: AML, anaplastic large cell lymphoma, brain tumor, and non-Hodgkins lymphoma. The chemotherapy protocols for these cases are listed in Appendix 3.

To obtain the 26, sex and age matched, controls we used patients presenting to the VCU Pediatric Dental Clinic for routine checkups. The researcher looked at the day's schedule for sex and age matched control subjects to previously examined case subjects. By the end of the study, in order to meet time constraints, one more male case subject and one fewer female subject was examined. This was due to scheduling constraints with a goal to match age over sex.

In the case group there were 11 males and in the control group there were 12 males (Table 2). In the case group the average age was 12.8 years (SD=2.6) and in the control group the average age was 13.3 years (SD=2.5) and the two ages were equivalent to within one year (P = 0.2388).

Description of Subjects

Overall, 56% were males and the average age of the cases and controls was 13 (SD=2.5, range = 9 to 18). The most common race category was African American (60%), followed by Caucasian (37%). Additionally, there was one Asian and one "other." Hispanics comprised 8% (n=4). The cases were more likely to have been hospitalized for a prolonged fever episode (31% vs. 8%, P=0.0299) but no more likely to report a latex allergy (4% overall, P=0.0912) or other allergies (17% overall, P=0.2676).

Only one control patient reported any serious problems associated with any previous dental treatment. There was no difference between the cases and controls on whether they had always had fluoride in their water (85% vs 88%, P>0.6), the number of dental exams per year (mean = 1.7 vs 1.7, P=1), whether they always used tooth paste with fluoride (92% vs 92%,

P=1), whether they used a fluoride mouth rinse (38% vs 35%, P>0.7), whether they flossed (69% vs 46%, P = 0.0905), or whether they had jaw clicking, etc. (8% vs 8%, P=1). All cases and controls reported that they brushed their teeth but the case group reported brushing their teeth more often (mean = 2.03 vs 1.61 per day, P=0.045). Both groups reported snacking between meals.

Developmental defects of the enamel

The primary outcome was the number of developmental defects in the enamel on the buccal, lingual and occlusal surfaces of #20 and #29. The DDE ratings were compared between the primary rater and the secondary rater to assess agreement. When the secondary rater examined the photographs he discovered the lingual surface could be not be accurately accessed via the photographs. Since the secondary rater could not assess the lingual surface, these scores were not included in the assessment of agreement.

Table 3 shows that there were a total of only 13 discrepancies out of 206 surfaces compared. The two raters agreed 94% of the time and the Kappa agreement was statistically significant (88%, P<.0001).

The DDE for each tooth and surface is summarized in Table 4. As may be seen, there is no difference between the teeth and so Table 5 summarizes the results by surface. As may be seen, the lingual surface has fewer abnormalities than either the buccal or occlusal surfaces. Analysis showed that the Buccal and Occlusal surfaces had statistically similar results. Table 6 depicts this analysis in that nominally there are more normal Buccal and Occlusal surfaces in the case group (73%) vs. the controls (59%). Hypoplasias were nominally higher in the control group (7%) vs. the cases (1%).

The primary comparison is between the cases and controls and the summary of these results appears in 7. Note that nominally there are more normal surfaces in the cases than in the controls (80% vs 70%) and that there are nominally fewer hypoplasias in the cases (5% vs 13%). Two analyses were considered, the first considered DDE as a binary outcome (normal vs abnormal) and the second considers DDE as a numeric score ranging from 0=normal to 3=hypoplasia. In the case of DDE as a binary outcome, a repeated-measures logistic regression included the following factors in the model: Case versus control, sex, age, tooth, and surface. There was no differences due to sex (P>0.4), age (P>0.2) or between the cases and controls (P=0.4517). There was no difference between the two teeth (P>0.5) but there was a difference between the three surfaces (P=0.0002). Tukey's multiple comparison procedure indicated that the probability of a defect on the lingual surface (6.6%) was lower than the probability of a defect on the buccal or occlusal surfaces (34.3% and 35.4%, respectively). There was no difference between the buccal and occlusal surfaces.

Considering DDE as a numerically scored value (0 to 3), a repeated-measures mixed-model ANOVA included the same factors in the model plus it considered that the group differences may vary by surface. The results indicated that there was a sex difference (P=0.0322). Females had a higher mean of 0.79 (95% CI = 0.47 to 1.10) than did the male mean of 0.32 (95% CI = 0.04 to 0.60). There was no age difference (P=0.122) and again there was difference in DDE depending upon the surface (P<.0001). Although there was no overall group difference (P=0.2356), the size of the group difference did depend upon the surface (P=0.0178). shows the estimated mean for each group and surface. For the buccal surfaces, the cases were nominally lower (P=0.0680) and there is no evidence for a case-control difference on the lingual surfaces (P>0.9).

Cancers and Leukemia

In this section, we describe the defects in two groups of cases. The two case groups are those with cancers (Wilm's tumor, brain tumor, anaplastic large cell lymphoma) and those with leukemia (ALL of all types, and AML). Table 9 describes the number of teeth with each of the conditions listed for the cancer cases, the leukemia cases, and the controls. Wilcoxon's nonparametric test indicated that the three groups were not significantly different on any of the variables (P>0.05).

Discussion

Previous studies [19] [5] [20] [21] have proven that chemotherapeutic agents used to treat childhood cancers resulted in a higher prevalence of various developmental defects in teeth because they were used during a period of time associated with the development of teeth. In this study we were attempting to confirm this by focusing on enamel developmental defects.

We were able to eliminate the majority of potential confounders prior to examination and the most common reasons for not participating in the study were scheduling availability, past orthodontic treatment, root canal treatment, and previous trauma to the area. Even though three subjects noted they did not live in a fluoridated water community they were included due to the fact that they were using fluoridated tooth paste and also due to the halo effect.

The number of participants were age and gender matched in most cases except for one more male in the case group and one more female in the control group. This was due to time constraints for this study in concert with scheduling constraints of subjects. The primary goal was to match on age over gender.

Initial analysis showed that the case group presented with more demarcated opacities than the control group, and the control group presenting with more diffuse opacities and hypoplasia than the case group. However, there were no statistically significant differences between the two groups. Thus the initial hypothesis appears to have not been confirmed, however the results are inconclusive due to one or more of the reasons noted below. In any case control study with negative results, there are four explanations for the findings.

1. There is no difference between the two groups. In the end both case and control groups showed statistically similar results when it comes to enamel formation. This may be due

to the fact that multiple chemotherapeutic protocols were used to treat case subjects. It is possible that the volume and/or type of chemotherapeutic agent used to treat the cancers may have different effects on enamel formation. This study did not take this into account.

2. Insufficient sample size and power to document a difference. Though there was a goal to include more case subjects in the study however, there were exclusion criteria and scheduling constraints that had to be met. This may have presented a bias in the data collection that may have been reduced had the subject populations been larger.
3. There was a bias in the case subject collection methods. Only those subjects that were able to make their appointments, and passed the exclusion criteria, were recruited. For example, subjects that presented with any syndromes were excluded. This may have excluded subjects that were exposed to different systemic or environmental factors, which may have resulted in an alternative conclusion.
4. There was a bias in the control subject collection methods. Only those subjects that were available and present at a dental examination were included in the control group. It is possible the results may have been different had the control subjects come from a more non-specific control population.

It should be noted that the lack of finding a difference between the case and control populations does not prove there is no difference. This study has simply not shown a difference between the two study groups. Further research is warranted. Given the reasons above, future studies should include a larger case subject population, use a longer period of data collection, and cover a larger population of control patients – for example, ones collected not only at dental

checkup exams. Additionally, future studies should consider using less exclusion criteria since this limited the available study population. A study whereby cases were identified prospectively while they were in the hospital for treatment could have fewer subjects excluded.

One aspect of the statistical analysis that was found to be statistically significant was in the realm of oral hygiene. All groups reported brushing their teeth but the case group reported brushing their teeth more often (mean = 2.03 vs 1.61 per day, P=0.045). While the control group did present more diffuse opacities, statistical analysis shows a non-significant difference between the two groups. With that said, it is likely that a larger study would have proved that an inadequate frequency of oral hygiene habits can cause diffuse opacities associated with decalcified areas. However, one may conclude that oral hygiene habits have a more significant impact on enamel formation than the effects of chemotherapeutic agents.

The general consideration concerning the etiology of opacities is that tooth morphogenesis is affected due to a harmful exposure during its mineralization phase. The fact that case group subjects present 81% normal enamel may imply that the regimen or protocols of chemotherapy are strong enough to eradicate the disease but are not harmful to secondary structures such as tooth enamel. However, future studies against larger populations may prove otherwise.

Though previous studies have shown a higher prevalence of development defects of the enamel organ in patients treated with chemotherapeutic agents, in this study these abnormalities were not observed to be statistically different than a control population. However, its possible study methods may have presented bias into the results.

Conclusion

In this study we were attempting to prove the hypothesis that chemotherapeutic agents used to treat different forms of cancer can cause developmental defects during enamel formation. This study did not observe that enamel was statistically effected differently between the case and control groups; although previous studies have shown otherwise. However, it is possible study methods may have presented bias into the results.

It should be noted that the lack of finding a difference between the case and control populations does not prove there is not a difference between the two groups. This study has simply not shown a statistical difference between the two specific study groups. Further research, against a larger study population, may prove otherwise.

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Tables

Table 1 Modified DDE Index

Code	Type of defect	Definition
0	Normal	
1	Demarcated opacities	The demarcated opacity presents a normal thickness of enamel with a surface intact, but there is a change in the translucency of enamel, of varying degree. This translucency is demarcated from the adjacent normal enamel with crisp and clear limits, and may present a white, beige, yellow or brown.[17]
2	Diffuse opacities	Diffuse opacity is also an abnormality involving a change in the translucency of enamel, of varying degree, and variable coloration as demarcated opacity. However, there is no clear boundary between adjacent normal enamel and diffuse opacity, and may present clinically in linear form or on plates, or have a confluent distribution.[17]
3	Hypoplasia	Hypoplasia is defined as quantitative defect of enamel visually and morphologically identified as involving the surface of enamel (an external defect) and associated with reduced thickness of enamel. The defective enamel may occur as (a) shallow or deep pits arranged horizontally in a linear fashion across the tooth surface or generally distributed over the whole or part of the enamel surface; (b) the defective enamel may occur as small or large, wide or narrow grooves; (c) in some instances there may be partial or complete absence of enamel over small or considerable areas of dentine.[12]
4	Other defects	If any defect does not fall into these categories, they were scored as others.

Table 2 Case-Control Matching

	Count	
Age	Case	Control
	Females	
9	2	0
10	2	2
11	1	0
12	0	1
13	4	3
14	1	1
15	1	2
16	0	1
17	0	2
	Males	
9	1	1
10	1	2
11	1	1
12	3	2
13	1	2
14	3	3
15	1	1
16	1	0
17	2	1
18	1	1

Table 3 Agreement between the primary and secondary examiner on the DDE index

	Primary Rater				
Secondary Rater	Normal	Demarcated opacities	Diffuse opacities	Hypoplasia	Total
Normal	133	7	0	0	140
Demarcated opacities	0	16	4	0	20
Diffuse opacities	0	0	17	0	17
Hypoplasia	2	0	0	27	29
	135	23	21	27	206

Exact agreement = 94%, Chance corrected Kappa agreement = 88%, P<.0001.

Table 4 DDE for each tooth location by cases and controls

		Cases		Controls	
Location	DDE	%	(n)	%	(n)
20 Buccal	Normal	77	(20)	58	(15)
	Demarcated opacities	8	(2)	4	(1)
	Diffuse opacities	8	(2)	23	(6)
	Hypoplasia	8	(2)	15	(4)
20 Lingual	Normal	92	(24)	92	(24)
	Demarcated opacities	4	(1)	0	(0)
	Diffuse opacities	4	(1)	8	(2)
	Hypoplasia	0		0	
20 Occlusal	Normal	73	(19)	58	(15)
	Demarcated opacities	15	(4)	12	(3)
	Diffuse opacities	4	(1)	12	(3)
	Hypoplasia	8	(2)	19	(5)
29 Buccal	Normal	72	(18)	58	(15)
	Demarcated opacities	16	(4)	4	(1)
	Diffuse opacities	4	(1)	19	(5)
	Hypoplasia	8	(2)	19	(5)
29 Lingual	Normal	96	(24)	92	(24)
	Demarcated opacities	4	(1)	0	(0)
	Diffuse opacities	0	(0)	8	(2)
	Hypoplasia	0		0	
29 Occlusal	Normal	68	(17)	62	(16)
	Demarcated opacities	20	(5)	12	(3)
	Diffuse opacities	8	(2)	4	(1)
	Hypoplasia	4	(1)	23	(6)

Table 5 DDE for each surface by cases and controls

		Cases		Controls	
Surface	DDE	%	(n)	%	(n)
Buccal	Normal	75	(38)	58	(30)
	Demarcated opacities	12	(6)	4	(2)
	Diffuse opacities	6	(3)	21	(11)
	Hypoplasia	8	(4)	17	(9)
Lingual	Normal	94	(48)	92	(48)
	Demarcated opacities	4	(2)	0	(0)
	Diffuse opacities	2	(1)	8	(4)
	Hypoplasia	0		0	
Occlusal	Normal	71	(36)	60	(31)
	Demarcated opacities	18	(9)	12	(6)
	Diffuse opacities	6	(3)	8	(4)
	Hypoplasia	6	(3)	21	(11)

Table 6 Percentage of Surfaces Affected

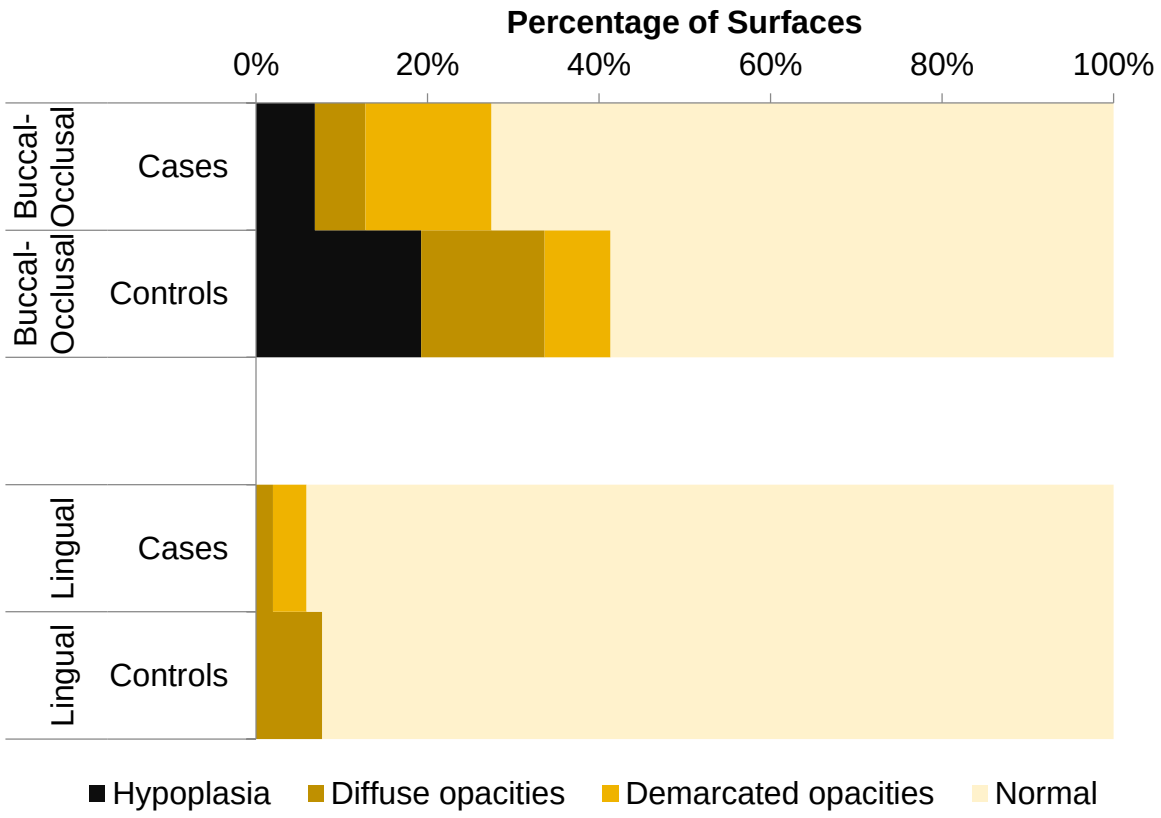

Table 7 DDE by cases and controls

	Cases		Controls	
DDE	%	(n)	%	(n)
Normal	80	(122)	70	(109)
Demarcated opacities	11	(17)	5	(8)
Diffuse opacities	5	(7)	12	(19)
Hypoplasia	5	(7)	13	(20)

Table 8 DDE scores by cases and controls

	DDE			
Surface	Estimated Mean	95% CI		P-value[1]
	Cases			
Buccal	0.561	0.239	0.883	0.0680
Lingual	0.169	-0.153	0.490	0.9533
Occlusal	0.561	0.239	0.883	0.1347
	Controls			
Buccal	0.982	0.663	1.301	
Lingual	0.155	-0.164	0.474	
Occlusal	0.905	0.586	1.224	

[1] The cases and controls were not significantly different by repeated-measures ANOVA (P>0.2) but the group difference varied by surface (P=0.0178). P-value compares the case and controls separately by each surface.

Table 9 Counting teeth by cancer, leukemia, and controls

Number of teeth with …	Cancer %	Cancer (n)	Leukemia %	Leukemia (n)	Controls %	Controls (n)	P-value
all surfaces normal							0.1980
0	38	(3)	17	(3)	42	(11)	
1	38	(3)	17	(3)	4	(1)	
2	25	(2)	67	(12)	54	(14)	
demarcated opacities							0.2562
0	50	(4)	72	(13)	81	(21)	
1	25	(2)	17	(3)	8	(2)	
2	25	(2)	11	(2)	12	(3)	
diffuse opacities							0.0539
0	63	(5)	94	(17)	62	(16)	
1	38	(3)	0	(0)	15	(4)	
2	0	(0)	6	(1)	23	(6)	
hypoplasia							0.1625
0	75	(6)	94	(17)	73	(19)	
1	13	(1)	6	(1)	4	(1)	
2	13	(1)	0	(0)	23	(6)	

Appendices

Appendix 1: Health History Form

Comparing tooth enamel disturbances in a pediatric population that received chemotherapy treatment to age-matched controls from the VCU Pediatric Dentistry clinic.

HEALTH HISTORY FORM

Patient's Name **Date of Birth** **Sex** **Race**

IN THIS HEALTH HISTORY FORM, "YOU" ALWAYS REFERS TO THE RESEARCH PARTICIPANT. IF YOU ARE A LEGALLY AUTHORIZED REPRESENTATIVE, PLEASE REMEMBER THAT "YOU" REFERS TO THE STUDY PARTICIPANT. **Answer all questions by circling Yes (Y) or No (N) All responses are kept confidential**

Medical and Dental History

Question	Answer	Description
Have you ever been diagnosed with any disease by a medical professional? If yes, describe condition and any treatments received. Please list all diseases if more than one. Also list date or year of diagnosis.	**Y N**	
Have you ever been hospitalized for a prolonged fever episode? If yes, please describe and indicate if treated with antibiotics	**Y N**	
Are you Allergic to or have you had any adverse reactions to:		
Latex or Rubber Products?	**Y N**	
Other allergies or reactions? Please, list.	**Y N**	
Have you had any serious problems associated with any previous dental treatment?	**Y N**	
Have you always have fluoride in your water?	**Y N**	
How often do you get periodical dental exams?		
Have you always used fluoridated tooth paste?	**Y N**	
Do you use any fluoride mouth rinse?	**Y N**	
Do you brush your teeth? How often?	**Y N**	
Do you floss your teeth? How often?	**Y N**	
Have you had any injuries or trauma to your teeth?	**Y N**	
Have you had braces?	**Y N**	
Have you had a root canal done?	**Y N**	
Do you have clicking or popping of jaw joint, pain near ear, difficulty opening mouth, grind or clench teeth? If yes, please describe.	**Y N**	
Do you snack between meals? If yes, please describe typical snacks.	**Y N**	

I understand the importance of a truthful Health History to assist the doctor in this research. I have had the opportunity to discuss my Heath History with my doctor.

_____ _____ _____

Date Signature of Person Completing Health History Doctor's Initials

Appendix 2: Chemotherapy protocols for the cases

Diagnosis	Chemotherapy protocol	N
ALL	8CUSTOM	1
ALL	AALL0331	5
ALL	POG 9605	1
ALL	POG 9905	1
ALL	POG 9905 Regimen A	1
ALL	POG9605	1
ALL	POG9605,AALL0232,AALL0031	1
AML	POG 9822	1
Anaplastic Large Cell Lymphoma (Murphy's Stage III)	POG 9315	1
B-lineage ALL	AALL0331	1
B-lineage ALL	POG 9605	1
Brain Tumor	POG 9233	1
Non Hogkins Lymphoma	POG 9605	1
Pre B ALL	AALL0331	2
Pre B ALL	PCP 1991	1
Pre B ALL	POG 9904 Regimen D	1
Wilm's Tumor	DD4A	3
Wilm's Tumor	EE4A	2

Vita

Marcela Roxana Mujica was born in Ica, Peru on September 3, 1975. In 2009 she became a United States Citizen. She earned a Doctor of Dental Surgery degree from San Luis Gonzaga National University in Ica, Peru in 1999. She earned a Doctor of Dental Medicine degree from the University of Pennsylvania School of Dental Medicine in 2012. She entered into the Pediatric Dentistry Residency Program at Virginia Commonwealth University's School of Dentistry in 2012.