6-2007

Salivary Characteristics in 3 and 4 year-old Children with and without Visible Caries

Gloria Yañez

LOMA LINDA UNIVERSITY
School of Dentistry
in conjunction with the
Faculty of Graduate Studies

Salivary Characteristics in 3 and 4 year-old Children with and without Visible Caries

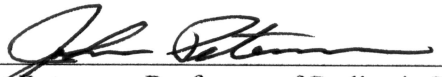
by

Gloria Yañez, D.D.S.

A Thesis submitted in partial satisfaction
of the requirements for the degree of
Master of Science in Pediatric Dentistry

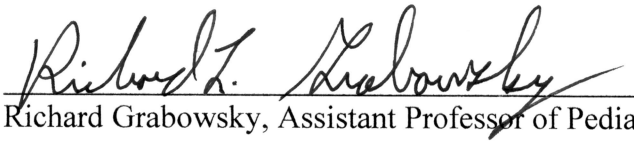
June 2007

Each person whose signature appears below certifies that this thesis in his/her opinion is adequate, in scope and quality, as a thesis for the degree of Masters of Science.

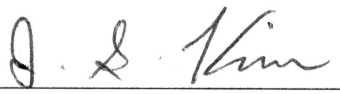


,Chairperson

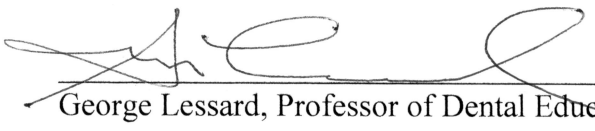
John Peterson, Professor of Pediatric Dentistry



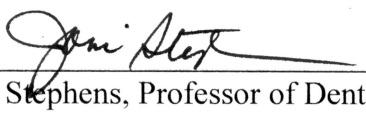
Richard Grabowsky, Assistant Professor of Pediatric Dentistry



Jay Kim, Professor of Statistics



George Lessard, Professor of Dental Education Services



Joni Stephens, Professor of Dental Hygiene

ACKNOWLEDGEMENTS

First and foremost, I would like to express my deepest gratitude to everyone who helped me along the way in completing this research project, especially to the members of my research committee: Dr. John Peterson, Mrs. Joni Stephens, Dr. Richard Grabowsky, Dr. George Lessard, and Dr. Jay Kim. An endeavor like this is never easy and I could not have accomplished this without their interest, support, or encouragement, so my heartfelt thanks to each and every one. I am also very grateful to Debbie Iacono for her help with the donation of the saliva test kits from GC America, INC. I would like to thank Denise Sinohui from the San Bernardino Preschool Services Department for facilitating and providing the support needed at all of the eight individual Head Start schools we visited. I would also like to thank the Head Start Site Supervisors and their staff for helping us with the children's participation and the consent and questionnaire forms. A very special additional thank you goes to Dr. Richard Grabowsky for shepherding the research project through many of our visits at the Head Start schools and his help during the saliva collection process. I would also like to thank Drs. Todd Milledge, Bonnie Nelson, and Joseph Curtin for their help when Dr. Grabowsky was not available. Finally, I would also like to thank Drs. Huan Lu, Tom Rodgers, and Brian Black for their guidance and feedback on this project. Thanks again.

CONTENTS

TABLES

FIGURES

ABBREVATIONS

USS	Unstimulated Saliva
SS	Stimulated Saliva
USSFR	Unstimulated Salivary Flow Rate
SSFR	Stimulated Salivary Flow Rate
CAT	Caries-Risk Assessment Tool
UAB	Upper Anterior Buccal
LALi	Lower Anterior Lingual

ABSTRACT OF THE THESIS

Salivary Characteristics in 3 and 4 year-old Children with and without Visible Caries

by

Gloria Yañez, D.D.S.

Master of Science, Advanced Education Program in Pediatric Dentistry
Loma Linda University, June 2007
Dr. John Peterson, Chairperson

PURPOSE: To compare salivary characteristics in 3 and 4 year-old Head Start children with and without visible caries.

METHODS: This case-control study was performed on 84 healthy 3 and 4 year-old Head Start children. After consent and inclusion criteria were met, each child was screened and assigned to one of two test groups based on their caries status. Group I consisted of children with no visible evidence of caries, although non-visible caries may have been present. Group II consisted of children with visible evidence demonstrating three or more carious lesions. Each child's unstimulated and stimulated whole saliva samples were collected and analyzed using a saliva test kit. The unstimulated saliva samples were analyzed for consistency, flow rate (USSFR), and pH. The stimulated saliva samples were analyzed for flow rate (SSFR), pH, and buffering capacity. The statistical methods used were the Mann-Whitney U test and 2 x 2 χ^2 contingency test at the significance level of $\alpha = 0.05$ to evaluate statistical differences.

RESULTS: There were no statistically significant differences in any of the salivary characteristics between the groups with and without visible caries. Nor was there a statistically significant difference between the genders within the individual groups.

However, there was a statistically significant difference between the 3 and 4 year-olds, with the 4 year-olds in Group I having statistically higher USS pH ($p=0.005$) and SS pH ($p=0.012$), and the 4 years-olds in Group II having statistically higher SSFR ($p=0.011$). Finally, the findings of the combined groups were similar to the individual groups, with no statistically significant differences between the genders and the 4 year-olds having statistically higher results in USS pH ($p=0.005$), SSFR ($p=0.011$), and SS pH ($p=0.006$). CONCLUSIONS: There were no differences between the groups with and without visible caries, although there were some differences in the combined and individual groups.

CHAPTER ONE

INTRODUCTION

An understanding of saliva and its role in the wellness of oral tissues is necessary to assess existing and potential oral health problems. Its scope should be part of the overall picture needed to determine the benefits of prevention or dental treatment. Any chronic reduction in salivary flow not only can affect the quality of life in normal every day events such as eating, swallowing, speech, and taste but can also result in the rapid deterioration of oral tissues, leading to trauma, infection, and progressive dental caries.[6, 21, 34, 40] Saliva is a vital body fluid composed of cellular, molecular, organic, and inorganic constituents with salivary flow as a key factor affecting its composition.[15, 32, 35, 40] It is essential for the maintenance of healthy oral tissues.

Saliva is a dynamic fluid that protects teeth and the oral mucosa with the ability to cleanse, dilute, lubricate, neutralize, and buffer. It regulates pH and the concentrations of its constituents by the type and duration of stimulation. As an ion reservoir it facilitates the remineralization of teeth. It also promotes antimicrobial actions and mechanisms that help control and eliminate bacteria.[6, 15, 32, 35, 40]

Low salivary flow may predispose children to a higher caries risk, especially those of preschool age.[1, 30] Many of these preschool children are in Head Start programs and have not had their first comprehensive oral examination or become part of a dental home. Early dental visits that begin with a caries risk assessment offers the opportunity

to educate and inform parents about their children's oral health.[2, 19, 36] Saliva testing may be an important benefit to many of these children and if possible, these tests should be performed before the planning of any restorative therapy.[10] Thus, by evaluating the caries resistant properties of saliva as a supplement to clinical findings, the intensity and progression of dental disease can be reduced or eliminated. [13, 24]

Literature Review

Whole saliva is a complex mixture of all the secretions in the mouth. Most of these secretions originate from the major and minor salivary glands. The three major paired salivary glands consist of the parotid, submandibular, and sublingual glands.[32, 40] The largest of these structures are the parotid glands whose secretions are mostly serous or watery in consistency. Next in size are the submandibular glands whose secretions are a mixture of mucous and serous fluids. This is followed by the mucous producing sublingual glands. The minor salivary glands are distributed throughout most of the buccal and labial mucosa, tongue and palate. Their secretions along with any other fluid producing sites are a viscous mixture of mucous and serous fluids.[32, 40]

As a result of the varied arrangement of the different types of salivary glands and their unique secretions, the distribution of salivary flow occurs unevenly as this complex mixture combines into a homogenous mix. [16, 23, 32, 40] Consequently, since the mouth is not a uniform environment there are many retentive sites where different types of bacteria can adhere and create ecosystems of colonization.[7, 23, 26] Thus, any reduction in salivary flow can result in the rapid overgrowth of pathogenic bacteria. [7, 32, 34] Fortunately, as a protective mechanism saliva tends to be a very selective growth medium.[7, 23] In addition,

the simple act of swallowing can result in the clearance of a large number of bacteria.[33] However, once the transmission of pathogenic infectious bacteria has occurred the resident microflora can easily be overwhelmed, especially if the energy sources for selective growth have changed. [7, 32, 34]

Whole saliva can be collected under resting or stimulated conditions by a number of simple noninvasive techniques. [21, 28] It can be readily analyzed for most of its constituents as well as its different innate properties, such as salivary flow rate, pH, and buffering capacity. The most commonly used reproducible techniques for collecting whole saliva are the draining, spitting, suction, or swab methods and the two most commonly used methods for inducing stimulated saliva are the masticatory and gustatory methods.[21, 28]

In measuring salivary flow, the standardization of saliva collection is very important because the volume can vary greatly within and between individuals. Also host and environmental factors can easily affect and reduce the rate of salivary flow.[21, 28] Birkhed and Heintze[6] reviewed various studies that compared the different techniques used in the collection of both resting and stimulated saliva and found good agreement and reproducibility between the techniques. They also found that the degree of stimulation influences both the flow rate and the proportion of secretions from each type of salivary gland. Flow rates can vary from almost no salivary flow during sleep, to copious amounts of saliva with stimulation.[15, 32, 40] In addition, the amount of secretions from the minor salivary glands during rest or lack of stimulation can exceed the secretions from the major salivary glands, while the reverse is found during stimulation. Thus, saliva consistency can change depending on the proportion of the individual gland secretions.[15]

The average unstimulated and stimulated salivary flow rate values found in adults, using various whole saliva collection methods are 0.3 ml/ min and 1.5 - 2.0 ml/ min respectively, both with wide normal ranges.[5, 15, 40] Also, the average maximum volume found for stimulated salivary flow rate was approximately 7 ml/ min.[15] By comparison, the average values found by Watanabe and Dawes[38] in five year-olds for unstimulated salivary flow rate (USSFR) is 0.22 ml/ min and 0.6 ml/ min for stimulated salivary flow rate (SSFR) by Andersson et al.[3] In addition, the SSFR studies by Andersson et al.[3] and Crossner [11] which included children from ages 5 to 15, found that the average flow rate increased with age up to 15 years-old and thereafter was the same as adults.[3, 5, 11] Moreover, those studies that have been done with infants and younger children have shown similar results with salivary flow rates increasing with age.[18, 22] For example in a USSFR study by Dezan et al.[18] using the suction method to obtain samples in a group of children between the ages of 18 to 42 months, the USSFR was higher in the 30 to 42 month-old group when compared to the 18 month old group. Although they found no statistical difference between the genders, boys tended to have higher flow rates than girls. In another USSFR study done with young children between 4 to 7 years of age, Bretz et al.[8] found that secretion rates were significantly higher in older children and agreed that boys had a higher USSFR than girls but not at a level of statistical significance. In an interesting study by Watanabe et al.[39] where they followed 30 five year-old children for two days, it was found that their estimated total saliva produced per day to be approximately 500 ml. They also found that the average USSFR was 0.26 ± 0.16 ml/ min and that the SSFR through eating and chewing different foods to be 3.6 ± 0.8 ml/ min with no statistical difference between the genders.[39]

Although the main function of stimulated saliva is to neutralize, buffer, and flush acids from teeth, its overall purpose is clearance.[16, 24] The main function of unstimulated saliva is to lubricate and provide antimicrobial effects. [16] The effect of increased salivary stimulation in producing fresh saliva together with the swallowing process results in faster clearance of dissolved substances, bacteria, and food debris. Since clearance occurs rapidly in areas of bulk saliva, due to the location of the larger salivary glands, different regions in the mouth are known to clear faster than others.[16, 17, 26] Generally, the lingual surfaces of the teeth are exposed to more watery secretions while the buccal surfaces are exposed mainly to the more viscous type, except where the secretions from the parotid glands wash the buccal surfaces of the upper posterior teeth.[32, 40] So in regards to a sucrose challenge, the most critical functions that stimulated saliva can provide for children are the flushing and buffering effects as well as the salivary clearance. [12, 21, 22] In addition, low salivary flow is correlated with slow salivary clearance.[16] Moreover, several studies have shown that caries experience has an inverse relation to salivary flow and buffering capacity. [6, 24, 33, 36]

Watanabe[37] evaluated the rates of salivary clearance at seven different sites in the mouths of twelve 5 year-old children. His findings for both groups showed that the salivary clearance was slowest in the upper anterior buccal (UAB) regions and fastest in the lower anterior lingual (LALi) regions. This suggests that certain areas of the primary dentition are more susceptible to caries, especially the upper anterior buccal regions. In contrast, Nevin and Walsh[29] found that clearance was slower in the interproximal spaces when compared to the maxillary anteriors. They also found the rate of salivary clearance varied greatly in the lower posterior regions, especially in the pit and fissure areas.

In a comparative study by Crossner et al.[12] which measured salivary clearance after a sugar challenge in 30 children between the ages of 3 and 15 versus 20 adults, it was shown that clearance takes longer in younger children and becomes more rapid with age. A similar study done by Hase[22] with 50 children between the ages of 3 and 15 versus adults, also showed that salivary clearance was comparatively slower in the younger children and increased with age. In addition, the younger children had lower salivary flow rates which may have been a factor in slower clearance.

Two very important physiological affects of salivary clearance are the swallowing frequency and the residual volume of saliva remaining after swallowing.[9, 16, 38] Both regulate the formation of the salivary film layer which covers the oral mucosa and the tooth pellicle.[25, 38] Since much of saliva generally persists as a coating or a film, its thickness is crucial with respect to clearance. Dawes [16] describes the process of salivary clearance as the removal of substances that do not bind to the oral surfaces. The important factors which affect clearance are the residual and maximum volumes of saliva and the unstimulated and stimulated flow rates.[16] He also suggested that the residual volume may be the reason why individuals differ in salivary clearance patterns, because individuals who swallow frequently and effectively can clear substances much faster by progressively diluting the residual volume of saliva. Collins and Dawes[9] determined from the residual volume of saliva and the calculated surface area of the mouth that the average salivary film thickness covering the teeth and the oral mucosa of adults was between 0.07 and 0.1 mm. Later, Watanabe and Dawes[38] determined the average film thickness in five year-old children to be 0.06 and 0.09 mm. Although there were similarities in film thicknesses, they found differences in the salivary flow rates and

residual volumes between adults and children. They also found that children had a decreased volume per swallow. Hence during a sucrose challenge, higher concentrations of dissolved acidic substances and bacteria could bind and accumulate in the salivary film and plaque layers. As a result, when the salivary film is at its thinnest and the clearance rate at its slowest, acids can diffuse towards the tooth surface.[14, 16, 27, 38] Lack of good oral hygiene can result in dense accumulation of plaque and bacteria.[22] This can subject teeth and gingival tissues to longer exposure of bacterial metabolites which can cause dental caries and gingival inflammation. Younger children are especially vulnerable to increased levels of plaque accumulation because of undeveloped oral sensory motor skills and inadequate awareness of oral hygiene.[22, 24]

Under resting conditions, the salivary pH is fairly constant and the pH of newly formed plaque tends to reflect the stimulated salivary pH and buffering capacity.[15] However, after a sugar challenge or exposure to fermentable carbohydrates, the salivary pH drops rapidly to a minimum before rising back to its resting pH. [20] The Stephan curve is the fall and subsequent rise of plaque pH over time after an exposure to fermentable carbohydrates.[20] Cariogenic bacteria in subjects with rapid salivary clearance have less exposure time to ferment carbohydrates, thus producing a shallow Stephen curve. Others with slower clearance produce a deeper Stephan curve, because lower plaque pH values can be reached.[16, 20] Increases in saliva volume with stimulated salivary flow causes the pH and the concentrations of salivary buffers to rise. This rise in volume also increases the swallowing frequency which facilitates the removal of bacteria and acidic substances.[15, 16] However, many of these acidic substances have different diffusion and binding properties which can allow them to concentrate more in plaque than saliva.

Overall, salivary pH and buffering capacity are dependent on salivary flow and clearance.[16, 20]

The buffering effectiveness or capacity of saliva to neutralize strong acidic or basic substances put in the mouth is generated by the concentrations of three main buffering systems. These are the bicarbonate, phosphate, and salivary proteins buffering systems.[6, 20, 24, 33,] In stimulated saliva, the parotid glands are the main producers of salivary flow and the chief buffering substance is bicarbonate.[15]

Diffusion of bicarbonate ions into plaque, especially during a sucrose challenge, helps to neutralize acids and limit a fall in plaque pH.[6] A serious consequence of low salivary flow is the diminished buffering capacity of saliva due to the overall reduction in the bicarbonate ion concentration.[6, 15, 24] In this case, the other buffering systems play an important role because they are not as dependent on stimulated flow.[24] The phosphate buffering system for example aids in maintaining the saturation concentration of calcium, phosphate, and hydroxyapatite ions during low or resting salivary flows which helps to reduce the demineralization of teeth.[24, 35] In a comparison study by Andersson et al.[4] where they measured the resting and stimulated saliva calcium and phosphate levels in children and adults, it was found that calcium levels were significantly lower in children with no significant difference in phosphate levels between adults and children. They also found the critical pH to be significantly higher in children. Moreover, SSFR were significantly higher in adults indicating that a normalized salivary pH level would be reached sooner.

It is well established that dental caries are caused by the demineralizing effects of acidic byproducts produced by cariogenic bacteria in plaque, especially when saliva

becomes less saturated with respect to calcium, phosphate, and hydroxyapatite ions.[14, 24, 25, 36] A plaque pH of 5.5 is the critical level where the tooth surfaces begin to demineralize.[20] Frequent sucrose challenges can drop and keep plaque pH well below the critical level. Thus plaque pH is a critical factor that can determine the initiation, progression, or repair of caries.[20] Low or impaired salivary flows and slow clearance rates can produce periods of prolonged exposure to low plaque pH which can reestablish the critical pH at a higher level.[14, 20]

Many salivary properties essential for caries and its prevention are connected with salivary flow and oral clearance.[16] Thus low salivary flows may predispose children to a higher caries risk, especially those of preschool age. Saliva testing could be an important benefit to many of these children. If possible, these tests should be performed before the planning of any restorative therapy.[10] Therefore by evaluating the caries resistant properties of saliva as a supplement to clinical findings, the intensity and progression of dental disease could be reduced or eliminated.

The purpose of this study was to compare salivary characteristics in 3 and 4 year-old Head Start children with and without visible caries. The specific aim of this study was to evaluate the use of whole saliva testing as a supplemental tool to a caries risk assessment and to determine whether it would be useful in a clinical setting before decisions of prevention and treatment are made.

Hypothesis

The null hypothesis is that there will be no statistically significant differences in the salivary characteristics of 3 and 4 year-old Head Start children with and without visible caries.

The alternative hypothesis is that there will be statistically significant differences in the salivary characteristics of 3 and 4 year-old Head Start children with and without visible caries.

CHAPTER TWO

MATERIALS AND METHODS

Figure 2.1 Saliva study materials.

Materials

- Saliva-Check Test Kits from GC America, INC.
  Contents Used:
  - 5.0 - 8.0 pH test strips
  - Saliva collection cup
  - Wax
  - Saliva dispensing pipette
  - Buffer test strips

- Denver XS-410 Balance- 0.01 mg sensitivity

- Digital timer

- Patient Bibs

- Nitrile Gloves

- Medicine cups

- Plastic Bag with following contents:
    - Oral-B® Child toothbrushes
    - Oral-B® Stages toothpaste
    - GUM® trial size floss
    - Spiderman & Disney Princess Stickers
    - Two small toys

Methods

This study was approved by the Loma Linda University Institutional Review Board. It was also approved by the administration of the Preschool Services Department of the San Bernardino County Head Start Centers. The Head Start program is a comprehensive child development program that focuses on assisting children from birth to age 5. Its main goal is to increase school readiness in young children of low income families. Eligibility for the Head Start program is income based and families must be at or below the federal poverty level.[30] The following eight Head Start Centers located throughout the city of San Bernardino were selected: Boys and Girls Club, Del Rosa, Highlands, Mill Child Development, Parks and Recreation, Waterman Gardens, Westside Plaza, and Westside Plaza Annex. The Site Supervisors or teachers at each Head Start Center distributed the saliva study information packets to the parents or guardians of the 3 and 4 year-old children in their classrooms. (See Appendix for a copy of the saliva study packet sent to parents.) Approximately 450 packets were distributed. Each packet consisted of an invitation letter with information on the study's purpose, a consent form, and two questionnaires concerning medical and dental history and dietary and oral hygiene in both English and Spanish. They also received an extra copy of the consent forms for their records. The parents or guardians who were interested in having their child participate were asked to return the completed consent form and questionnaires to

the center. In addition to parental consent, each child had to give assent and show willingness to participate in the study.

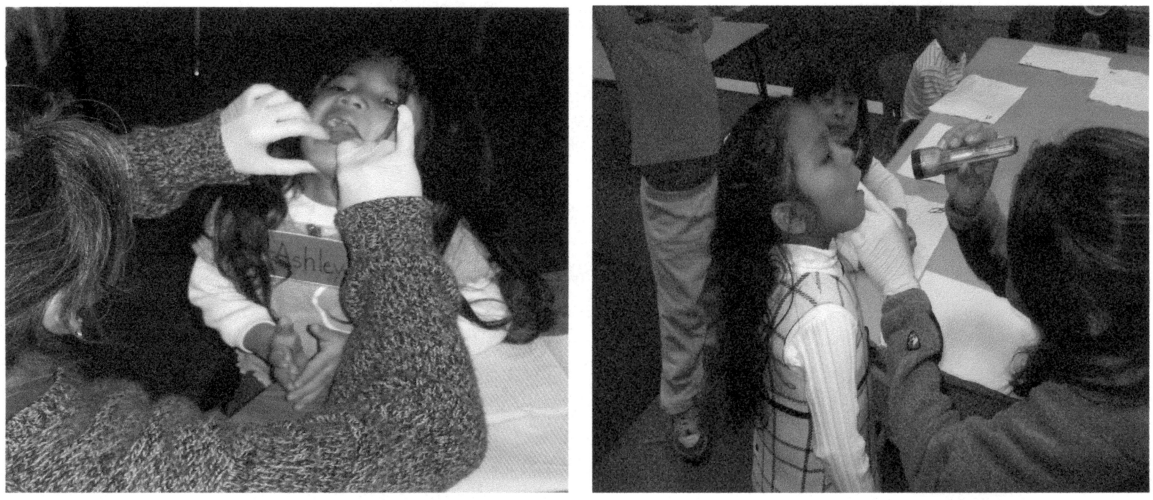


Figure 2.2 Dental Screenings.

From the 151 consent forms returned 105 children were screened. (Figure 2.2) The other 49 children were not screened because they were either absent, not willing to participate, or did not meet the preliminary inclusion criteria. Preliminary inclusion criteria included age, no systemic disease, no medications (prescription or over-the-counter), and the ability to spit and chew gum. The children who met the inclusion criteria were screened for caries status and restorations. Children who had any restorations were not selected as participants. In addition, those children with caries who had less than 3 visible carious lesions were also not selected. From the 105 children who were screened 84 were selected and became study participants. The 84 study participants were assigned to one of two test groups based on their caries status. Group I consisted of children with no visible evidence of caries, although non-visible caries may have been

present. Group II consisted of children with a minimum of three or more visible carious lesions. Although non-visible caries may have been present in each group, caries status was determined on only a visual examination of teeth. No radiographs were taken. All dental screenings were performed by the same examiner throughout the entire study. All saliva collections and analyses were performed under controlled conditions and took place on the school premises in a quiet classroom or designated area.

On the assigned days of screening and collection, the Head Start teachers were instructed not to allow the children to eat or drink anything for at least 1 hour after their school breakfast. All Head Start centers are required to serve breakfast at 8 and lunch at 11 AM. Therefore, all whole saliva samples were collected between 9:15 – 11 AM. A pilot study was conducted at the Westside Annex Head Start Center using the first 8 participants to ascertain if any modifications to the saliva collection procedure were warranted.

The recommended saliva collection procedure from the Saliva-Check Test kit from GC America, INC. was not used. In this study, unstimulated and stimulated whole saliva samples were collected using the spitting and masticatory saliva collection procedures. These standardized saliva collection procedures are frequently used in saliva studies to sample adults and older children and their typical collection period is either 5 or 10 minutes long.[2, 6, 11, 21,28] For this study the collection period was shortened to two minutes to accommodate the attention span of 3 and 4-year old children. The following contents from the Saliva-Check Test kit from GC America, INC. were used: saliva collection cup, paraffin wax, saliva dispensing pipette, 5.0 - 8.0 pH test strips, and a buffer test strip. An additional one ounce medicine cup was added to the test kit to

complete the sampling materials (Figure 2.1). The sample cups were prepared and weighed. See Figure 2.3.

Figure 2.3 Weighed determinations.

Figure 2.4 Saliva spitting demonstration.

Before any saliva samples were collected, the children were seated comfortably in a quiet classroom or designated area. A modeling demonstration with a simple explanation of the collection procedures was performed for the selected group of children (Figure 2.4). Following the demonstration, the children were asked to spit into a pre-weighed cup for 2 minutes using a timer. After the 2 minute collection period, the container with the USS was reweighed. The children were then given another pre-weighed cup with a piece of unflavored paraffin wax to chew. Both paraffin wax and cup were weighed together. The children were asked to chew the wax for 1 minute before any collection of the stimulated saliva. After 1 minute the children were asked to continue chewing the wax and to spit any saliva they produced into the pre-weighed cup for 2 minutes. See Figures 2.5 and Figure 2.6. At the end of the 2 minute collection period, the children were also asked to spit the chewed wax into the cup which was then reweighed. The weighed determinations of whole saliva samples were measured to determine the USS and SS flow rates. The specific gravity of water 1.000 g/ml (saliva's specific gravity is 1.0008 to 1.002)[40] was used to convert salivary flow rate from g/ min to ml/ min. All saliva samples were analyzed immediately after collection. The unstimulated saliva sample was analyzed for consistency and pH and the stimulated saliva sample was analyzed for pH, and buffering capacity. Salivary consistency was determined on a visual assessment of saliva viscosity. Unstimulated saliva was assessed as either ropey (sticky or mucous type consistency) or runny (watery consistency). USS and SS pH values were determined by using 5.0 - 8.0 pH indicating paper and the buffering capacity was determined by using a buffer colorimetric strip test. These simplified colorimetric methods for pH and buffering capacity were performed according

to the listed procedures in the Saliva-Check test kit and are estimated values. The buffering capacity was determined by leaving a few drops of saliva on three pH treated test pads located on the strip for 2 minutes. After 2 minutes, the color change of each test pad was compared to a colorimetric point scale. The total number of points (0-12) was interpreted as one of the following categories: 0 to 5 (very low buffering capacity), 6 to 9 (low), and 10 to12 (normal to high buffering capacity).

Figure 2.5 Spitting saliva into a sample cup.

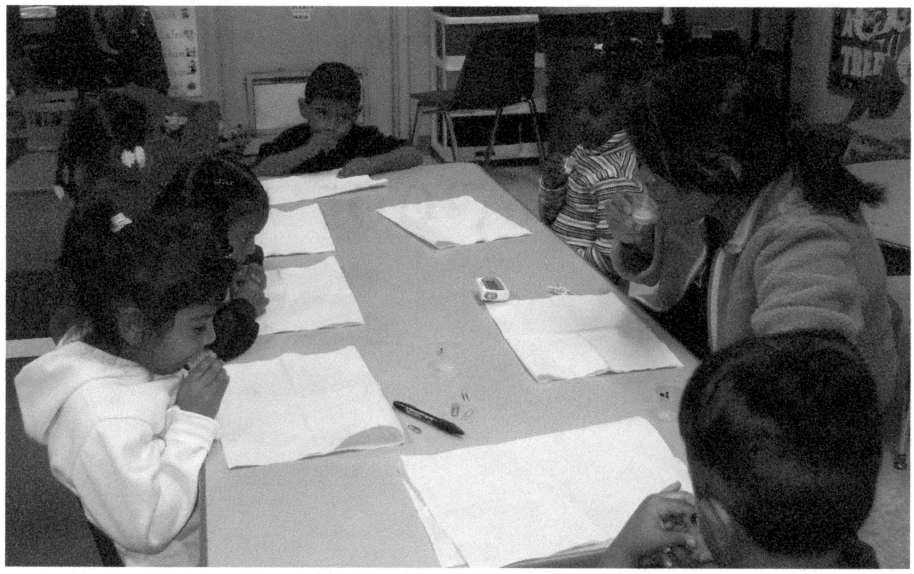


Figure 2.6 Group - Saliva sample collection.

Statistical Analysis

The estimated number of study participants was based at the significance level of $\alpha = 0.05$ and a 95% power in the statistical tests used to detect a minimum difference. The Mann-Whitney U non-parametric statistical test was used to detect any statistical differences in the salivary flow rates, pH, and buffering capacity between the two groups of children with and without visible caries. The same test was also used to test for any statistical differences in the salivary flow rates, pH, and buffering capacity of the combined and individual groups. The 2 x 2 χ^2 contingency non-parametric statistical test was used to determine any statistical differences in saliva consistency between the two groups of children as well as in the combined and individual groups.

CHAPTER THREE

RESULTS

Approximately 450 saliva study packets were distributed to the parents or guardians of the 3 and 4 year-old children at eight Head Start centers. A total of 151 completed saliva study packets were returned. From the 151 packets returned, only 105 children met the preliminary inclusion criteria to be screened. Forty-nine children were not included because they were either absent, not willing to participate, taking medications, or did not meet the age criteria. From the 105 children who were screened, 84 were selected and became study participants. A total of 84 participants completed the study and were included in the final data analysis. Thirty of these participants were 3 year-olds and 54 were 4 year-olds (Figure 3.1). All participants were assigned to one of the two test groups based on their caries status. Group I consisted of 51 children, 18 males and 33 females, with no visible evidence of caries, although non-visible caries may have been present. Group II consisted of 33 children, 10 males and 23 females, with three or more visible carious lesions. See Figure 3.2.

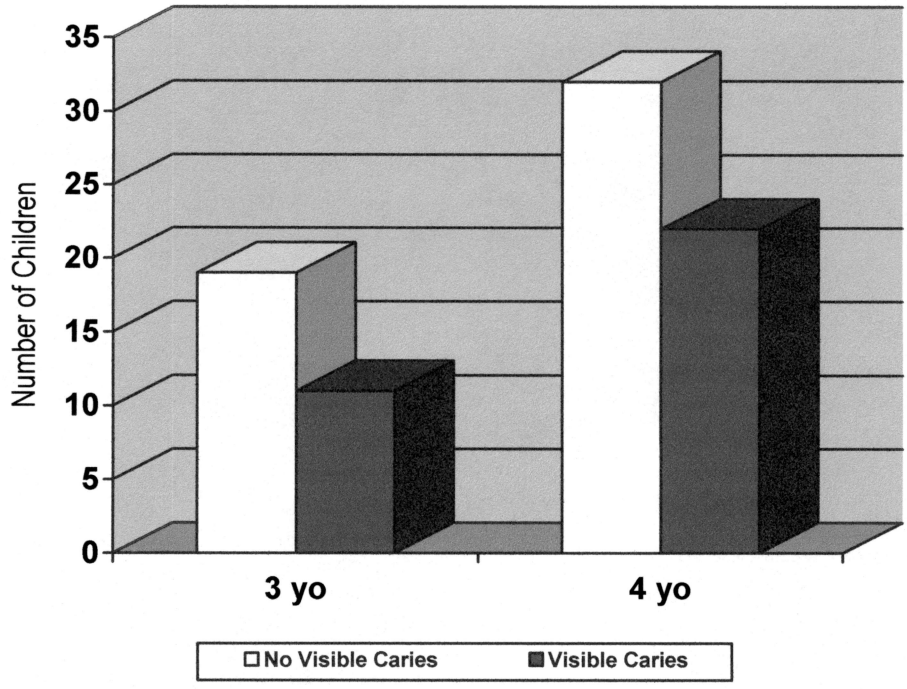


Figure 3.1 Saliva study 3 and 4 year-old participants.

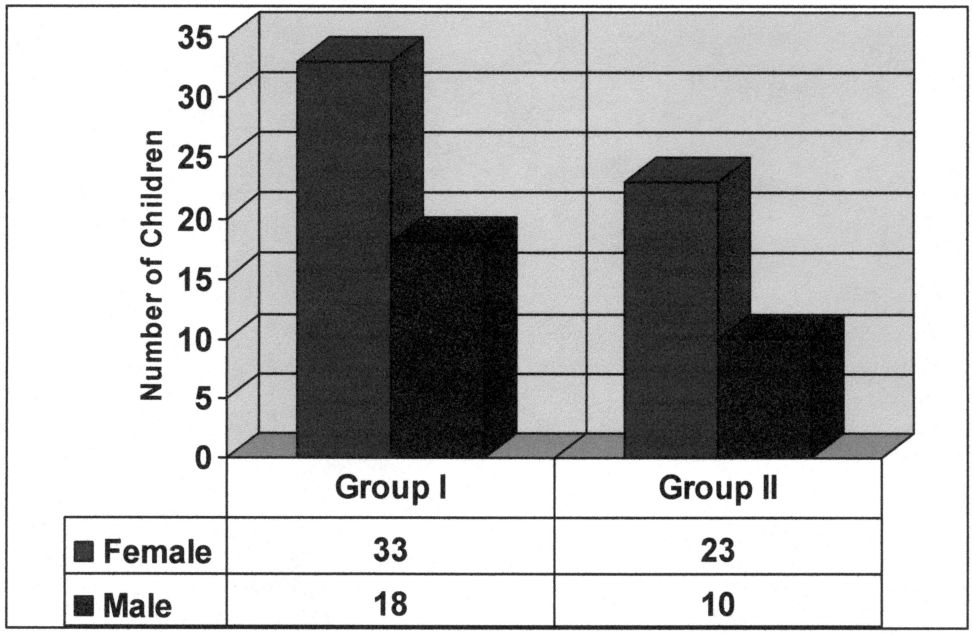


Figure 3.2 Group I (No Visible Caries) vs. Group II (Visible Caries)

Table 3.1 shows the mean value and standard deviation for each of the salivary characteristics evaluated in the combined groups. There was no statistically significant difference between the genders. Although females appear to have a higher stimulated salivary flow rate this difference was not statistically significant. In the age comparison, there was no statistically significant difference found in the USSFR, USS Consistency, or Buffering Capacity saliva characteristics between the 3 and 4 year-olds. However, 4 year-olds were statistically higher in USS pH ($p = 0.005$), SSFR ($p = 0.011$), and SS pH ($p= 0.006$).

Table 3.1 Combined Groups - Differences in Salivary Characteristics in Age and Gender

	Age Months	USSFR (ml/min)	USS pH	Consistency Runny	Consistency Ropey	SSFR (ml/min)	SS pH	Buffering Capacity
Combined Groups	(n = 84) 50 ± 6	(n = 82) 0.40 ± 0.29	(n = 80) 7.2 ± 0.5	(n = 51) 64%	(n = 29) 36%	(n = 81) 0.66 ± 0.47	(n = 80) 7.6 ± 0.3	(n = 77) 11 ± 1
Female	(n = 56) 49 ± 6	(n = 55) 0.40 ± 0.28	(n = 54) 7.2 ± 0.5	(n = 34) 63%	(n = 20) 37%	(n = 54) 0.72 ± 0.45	(n = 54) 7.7 ± 0.3	(n = 52) 11 ± 1
Male	(n = 28) 51 ± 6	(n = 27) 0.41 ± 0.32	(n = 26) 7.2 ± 0.4	(n = 17) 65%	(n = 9) 35%	(n = 27) 0.59 ± 0.51	(n = 26) 7.6 ± 0.2	(n = 25) 10 ± 1
3 years	(n = 30) 43 ± 3	(n = 29) 0.38 ± 0.32	(n = 27) 7.0 ± 0.5	(n = 16) 57%	(n = 12) 43%	(n = 29) 0.52 ± 0.40	(n = 29) 7.5 ± 0.4	(n =28) 10 ± 1
4 years	(n = 54) 53 ± 3	(n = 53) 0.42 ± 0.27	(n = 53) 7.3 ± 0.5	(n = 35) 67%	(n = 17) 33%	(n = 52) 0.73± 0.50	(n = 51) 7.7 ± 0.1	(n = 49) 11 ± 1

Values are mean ± SD. No statistically significant differences in any of the salivary characteristics between the genders. Age: 4 year-olds were statistically higher in USS pH ($p = 0.005$), SSFR ($p = 0.011$), and SS pH ($p=0.006$).

Table 3.2 shows the mean value and standard deviation for each of the salivary characteristics evaluated in Group I. There was no statistically significant difference between the genders. Although males showed a higher USSFR and females showed a higher SSFR they were not statistically significant. In the age comparison, the 4 year-olds were statistically higher in USS pH ($p = 0.005$) and SS pH ($p=0.012$). The 4 year-olds were also higher in USSFR and SSFR but there were no statistically significant

differences found. In addition, in comparing the USS Consistency between the 3 and 4 year-olds it appears that the 3 year-olds have a thicker saliva but not at a statistically significant level.

Table 3.2 Group I (No visible caries) - Differences in Salivary Characteristics in Age and Gender

	Age Months	USSFR (ml/min)	USS pH	Consistency		SSFR (ml/min)	SS pH	Buffering Capacity
				Runny	Ropey			
Female	(n = 33) 49 ± 5	(n = 32) 0.39 ± 0.29	(n = 31) 7.2 ± 0.5	(n = 21) 66%	(n = 11) 34%	(n = 31) 0.67 ± 0.44	(n = 31) 7.7 ± 0.3	(n = 31) 11 ± 1
Male	(n = 18) 51 ± 6	(n = 17) 0.44 ± 0.35	(n = 17) 7.2 ± 0.4	(n = 11) 65%	(n = 6) 35%	(n = 18) 0.56 ± 0.55	(n = 17) 7.7 ± 0.2	(n = 15) 11 ± 1
3 years	(n = 19) 43 ± 3	(n = 18) 0.40± 0.36	(n = 17) 7.0 ± 0.4	(n = 10) 56%	(n = 8) 44%	(n = 19) 0.52 ± 0.45	(n = 19) 7.5 ± 0.3	(n =18) 10 ± 1
4 years	(n = 32) 53 ± 4	(n = 31) 0.41 ± 0.27	(n = 31) 7.3 ± 0.4	(n = 22) 71%	(n = 9) 29%	(n = 30) 0.70 ± 0.50	(n = 29) 7.7 ± 0.1	(n = 28) 11 ± 1

Values are mean ± SD. No statistically significant differences in any of the salivary characteristics between the genders. Age: 4 year-olds were statistically higher in USS pH ($p = 0.005$) and SS pH ($p = 0.006$).

Table 3.3 shows the mean value and standard deviation for each of the salivary characteristics evaluated in Group II. There was no statistically significant difference between the genders. Although females showed a higher USSFR and SSFR and the males were higher at the USS pH and SS pH, the differences were not statistically significant. In the age comparison, overall the 4 year-olds were higher in all the salivary characteristics. However, there was only a statistically significant difference found in SSFR ($p = 0.011$). In addition, in comparing the USS Consistency between the 3 and 4 year-olds in the caries group it appears that the 3 year-olds have a thicker saliva but not at a statistically significant level. Table 3.4 shows the comparison between the groups. There was no statistically significant difference found in any of the salivary characteristics between the groups with and without visible caries.

Table 3.3 Group II (Visible caries) - Differences in Salivary Characteristics in Age and Gender

	Age Months	USSFR (ml/min)	USS pH	Consistency Runny	Consistency Ropey	SSFR (ml/min)	SS pH	Buffering Capacity
Female	(n = 23) 50 ± 7	(n = 23) 0.41 ± 0.28	(n = 23) 7.0 ± 0.5	(n = 13) 59%	(n = 9) 41%	(n = 23) 0.72 ± 0.48	(n = 23) 7.5 ± 0.3	(n = 22) 10 ± 1
Male	(n = 10) 47 ± 8	(n = 10) 0.37 ± 0.28	(n = 9) 7.2 ± 0.4	(n = 6) 63%	(n =3) 37%	(n = 9) 0.65 ± 0.37	(n = 9) 7.7 ± 0.1	(n = 9) 10 ± 1
3 years	(n = 11) 41 ± 4	(n = 11) 0.34 ± 0.28	(n = 10) 6.9 ± 0.6	(n = 6) 55%	(n = 5) 45%	(n = 10) 0.42 ± 0.27	(n = 10) 7.4 ± 0.4	(n =8) 10 ± 2
4 years	(n = 22) 52 ± 3	(n = 22) 0.43 ± 0.28	(n = 22) 7.1± 0.5	(n = 13) 65%	(n = 7) 35%	(n = 18) 0.83 ± 0.46	(n = 19) 7.7 ± 0.2	(n = 20) 11 ± 1

Values are mean ± SD. No statistically significant differences in any of the salivary characteristics between the genders. Age: 4 year-olds were statistically higher in SSFR (p = 0.011).

Table 3.4 Salivary Characteristics in Group I (no visible caries) and Group II (visible caries)

	Age Months	USSFR (ml/min)	USS pH	Consistency Runny	Consistency Ropey	SSFR (ml/min)	SS pH	Buffering Capacity
Group I	(n = 51) 49 ± 6	(n = 49) 0.41 ± 0.31	(n = 48) 7.2 ± 0.4	(n = 32) 65%	(n = 17) 35%	(n = 49) 0.63 ± 0.49	(n = 48) 7.7 ± 0.2	(n = 46) 11 ± 1
Group II	(n = 33) 47 ± 6	(n = 33) 0.40 ± 0.28	(n = 32) 7.1 ± 0.5	(n = 19) 62%	(n = 12) 39%	(n = 32) 0.70 ± 0.44	(n = 32) 7.6 ± 0.3	(n = 31) 10 ± 1

Values are mean ± SD. No statistically significant differences between Group I and Group II.

Family Dietary and Dental History Questionnaire – Table 3.5 shows the average responses to the dental information requested on the Family Dietary and Dental History Questionnaire returned in the saliva packets (n = 151). The average responses to the dietary portion of the questionnaire are as follows: number of Snacks per day – 2; number of Sugar Drinks per day – 2; number of times drink water per day – 4. Also, the average age reported for a child's first dental visit was 3-years (See Figure 3.3). Figure 3.4 shows the average age reported for the first dental visit of the 84 children who participated in the saliva study. It was also 3-years. The average responses to the dental portion of the questionnaire are as follows: 62% have regular dental visits, 64% get their fluoride from toothpaste, 40% use floss, 73% of the children brush their teeth alone, 94%

of the caregivers help their children brush in some way, and 46% of family members and 49% of caregivers have caries. In addition, of the 33 children in Group II (with visible caries), 15 mothers or caregivers reported that they have caries (45%). So aside from a socioeconomic risk factor, these responses indicate that there are many additional risk factors to consider as per the history portion of the AAPD Caries-Risk Assessment Tool.[2]

Table 3.5 Family Dietary and Dental History Questionnaire Responses

n = 151	Regular Dental Visits	Mom has Caries	Family has Caries	Mom helps with brushing	Child brushes Alone	Uses Floss
Yes	(n = 93) 62%	(n = 73) 49%	(n = 68) 46%	(n = 141) 94%	(n = 110) 73%	(n = 61) 40%
No	(n = 58) 38%	(n = 76) 51%	(n = 80) 54%	(n = 9) 6%	(n = 41) 27%	(n = 90) 60%

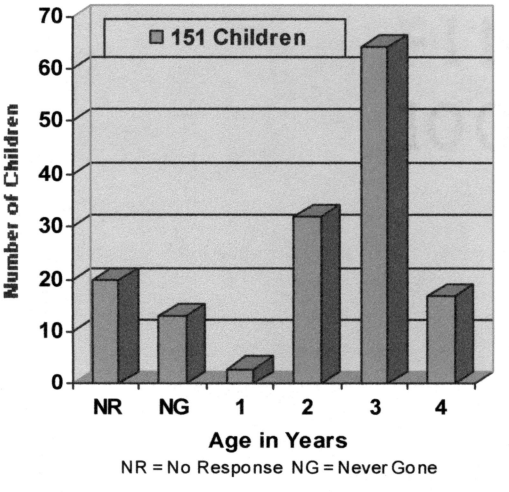


Figure 3.3 Average age of 1st Dental Visit.

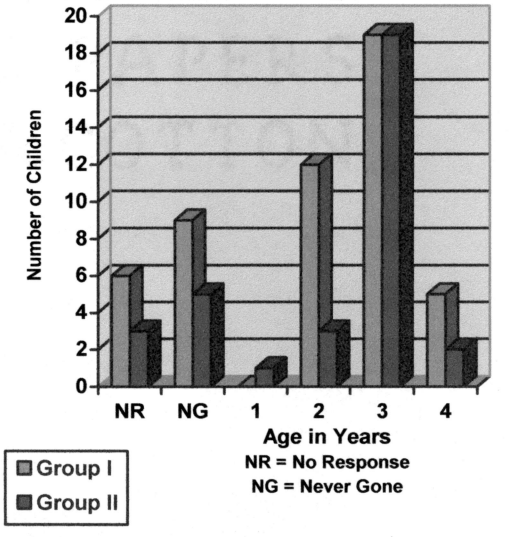


Figure 3.4 Average Age of 1st Dental Visit. 84 participants.

CHAPTER FOUR

DISCUSSION

This study investigated salivary characteristics in eighty-four 3 and 4 year-old Head Start children with and without visible caries from the San Bernardino, California area. Results relating to three properties of unstimulated saliva using the spitting method and three properties of stimulated saliva using the masticatory/spitting method were evaluated and reported. During the saliva collection procedures, most of the children who participated in this study did incredibly well in focusing and following instructions, although there were a few of the younger 3 year-olds who had somewhat of a difficult time trying to coordinate chewing wax and spitting into a small cup.

In measuring salivary flow rates under standardized conditions, the results of this study did not contradict any of the mean values reported in similar or related studies. The spitting method was used in this study to measure USSFR and the average ± SD reported is 0.40 ± 0.29 ml/min. Dezan et al.[18] used the suction method to measure USSFR in 42 month-old children and reported a mean ± SD of 0.66 ± 0.28 ml/min. In an earlier study by Watanabe et al.[39] they used the dripping method to measure USSFR of 5 year-old children and reported a mean ± SD of 0.26 ± 0.16 ml/min. For SSFR values, the masticatory/spitting method was used in this study and the reported mean ± SD is 0.66 ± 0.47 ml/min. Andersson et al.[4] used a similar method and reported a mean ± SD of 0.88 ± 0.52 ml/min as the SSFR for 6-12 year-old children. The average flow rate values in this

study are within the range of those reported in the other studies. However, any differences may be attributed to variations in salivary flows between the individual children and/or the result of any difficulties the children encountered with this type of saliva collection method. In addition, the results of this study supported some of what has been previously reported in similar studies in that salivary flow increased with age and that there is no significant difference between the genders. [3, 8, 11, 18, 22, 38] It also supported their findings in that males tend to have higher USSFR than females.[8, 22] However, in this study it was found that females had higher SSFR than males but not at the significant level.

According to the responses in this study's Family Dietary and Dental History questionnaire (n = 151), which evaluated other risk factors aside from the lower socioeconomic status, it was found that many of these children: lack a dental home (38%), have active caries (39%), their parents have caries (49%) and /or siblings have caries (46%), plus they eat an average of two sugar snacks and two sugar drinks per day. In addition, 9% of these children have not yet visited a dentist. Based on these findings it will be more difficult to identify those individual Head Start children who are at low risk of developing caries. So it is very important that early dental visits begin with a caries risk assessment to educate and inform parents about their children's oral health.[2, 19, 31, 36]

Based on the income criteria to participate in the Head Start program these children fall into the low socioeconomic category by the Caries Risk Assessment Tool (CAT) from the American Academy of Pediatric Dentistry (AAPD).[2] This criteria is a high risk factor for dental caries. However, the presence of one high predisposing risk indicator does not completely predict those children within this population who are at the

highest risk of getting dental caries.[27, 31] Since dental caries has a multifactorial etiology, other historical and clinical information is warranted to create a representative risk profile to better identify those individual children who are at the highest risk of developing dental caries. [2, 27, 31] Moreover, it would be difficult to convert any of these children into a lower caries risk status without identifying all the contributing risk factors.[2, 31] In general, as part of a initial caries risk assessment, all children enrolled in the Head Start program should have a detailed family dietary and dental history performed before a dental screening to help create a representative risk profile for each of these children. Furthermore, any risk assessment should be followed up by the establishment of a dental home.[1]

The specific aim of this study was to evaluate whole saliva testing in preschool children as a supplemental tool to a caries risk assessment and to determine if it might be useful clinically before the decisions of treatment and prevention are made. Although some saliva collection procedures may be difficult to perform on young children, there are some saliva tests which are easy to use and may help identify potential risk factors. Based on this study, it was found that salivary flow rates varied widely between individual children and it was difficult to assess gland function from a single flow rate determination. Therefore repeated measurements would be necessary to establish an individual's baseline flow rate.[27] In addition, it would be impractical to perform repeated flow rate measurements in younger children unless there are indications of an impaired salivary flow. Buffering capacity and pH on the other hand are inexpensive and simple tools which can help estimate a host response at any age without the use of complicated collection procedures. However, more studies are needed to determine if buffering

capacity and pH would be clinically useful. If possible, these tests should be performed as a supplement to a CAT, before the planning of any restorative therapy.[10]

CHAPTER FIVE

CONCLUSIONS

Based on the results of this study, which was limited to 3 and 4 year-old Head Start children, the following conclusions can be made:

- No statistically significant differences in any of the salivary characteristics between the groups of children with and without visible caries.
- No statistically significant differences between the genders within the individual or combined groups of children.
- The 4 year-olds in both the individual and combined groups showed statistically significant higher results in USS pH, SSFR, and SS pH when compared to the 3 year-olds.

REFERENCES

1. American Academy of Pediatric Dentistry. Oral Health Policy for the Dental Home. *Pediatr Dent.* Reference Manual 2005-2006, 27(7):18-19.

2. American Academy of Pediatric Dentistry. Use of a caries-risk assessment tool (CAT) for infants, children, and adolescents. *Pediatr Dent.* Reference Manual 2006-2007, 28(7):24-28.

3. Anderson R, Arvidsson E, Crossoer CG, Holm AK, Mansson B, Grahnen H. The flow rate, pH, and buffer effect of mixed saliva in children. *J Int Assoc Dent Child.* 1974;5:5-12.

4. Anderson P, Hector MP, Rampersad MA. Critical pH in resting and stimulated whole saliva in groups of children and adults. *Int J of Pediatr Dent.* 2001;11:266-273.

5. Becks H, Wainwright WW. Human Saliva XIII - Rate of flow of resting saliva of healthy individuals. *J Dent Res.* 1943;22:391-396.

6. Birkhed D and Heintze U. Salivary secretion rate, buffer capacity and pH. In: Tenovuo J, ed., *Human saliva: clinical chemistry and microbiology,* vol. I. Boca Raton: CRC Press, 1989. pp. 25-73.

7. Bowen W H. Salivary influences on the oral microflora. In: Edgar WM and O'Mullane DM, eds., *Saliva and oral health*, 2nd Edition. London: British Dental Journal, Thanet Press Limited, Margate, 1990. pp. 95-103.

8. Bretz WA, et al. Unstimulated salivary flow rates of young children. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* 2001;91:541-545.

9. Collins LMC, Dawes C. The surface area of the adult human mouth and the thickness of the salivary film covering the teeth and oral mucosa. *J Dent Res.* 1987;66:1300-1302.

10. Crall JJ. Development and integration of oral health services for pre-school children. *Pediatr Dent.* 2005;27(4):323-330.

11. Crossner CG. Salivary flow rate in children and adolescents. *Swed Dent J.* 1984;8:271-276.

12. Crossner CG, Hase JE, Birkhed D. Oral sugar clearance in children compared with adults. *Caries Res.* 1991;25:201-206.

13. Crossner, CG and Holm, AK. Saliva tests in the prognosis of caries in children. *Acta Odontol Scand.* 1977;35:135.
14. Dawes C. An analysis of factors influencing diffusion from dental plaque into a moving film of saliva and the implications for caries. *J Dent Res.* 1989;68:1483-1488.

15. Dawes C. Factors influencing salivary flow rate and composition. In: Edgar WM and O'Mullane DM, eds., *Saliva and oral health*, 2nd ed. London: British Dental Journal, Thanet Press Limited, Margate, 1990. pp.27-41.

16. Dawes C. Clearance of substances from the oral cavity implications for oral health. In: Edgar WM and O'Mullane DM, eds., *Saliva and oral health*, 2nd ed. London: British Dental Journal, Thanet Press Limited, Margate, 1990. pp. 67-79.

17. Dawes C, Watanabe S, Biglow-Lecomte P, and Dibdin GH. Estimation of the velocity of the salivary film at some different locations in the mouth. *J Dent Res.* 1989;68:1479-1482.

18. Dezan CC, Nicolau J, Souza DN, Walter LRF. Flow rate, amylase activity, and protein and sialic acid concentrations of saliva from children aged 18, 30 and 42 months attending a baby clinic. *Arch Oral Biol.* 2002;47:423-427.

19. Domenick Z, Fontana M, Lennon A. Clinical applications and outcomes of using indicators of risk in caries management. *J Dent Edu.* 2001; 65(10):1126-1131.

20. Edgar M and Higham SM. Saliva and the control of plaque pH. In: Edgar WM and O'Mullane DM, eds., *Saliva and oral health*, 2nd ed. London: British Dental Journal, Thanet Press Limited, Margate, 1990. pp. 81-96.

21. Flink H, Tegelberg A, Lagerlof F. Influence of the time of measurement of unstimulated human whole saliva on the diagnosis of hyposalivation. *Arch Oral Biol.* 2005;50:553-559.

22. Has JC. Influence of age and salivary secretion rate on oral sugar clearance. *Swed Dent J Suppl.* 1993;89:1-65.

23. Hay DI. Salivary factors in caries models. *Adv Dent Res.* 1995;9(3):239-243.

24. Lenander-Lumikari M, Loimaranta V. Saliva and dental caries. *Adv Dent Res.* 2000;14:40-47.

25. Lendenmann U, Grogan J, Oppenheim FG. Saliva and dental pellicle. *Adv Dent Res.* 2000;14:22-28.

26. Mcdonnell S. and Hector MP. The secretion and distribution of saliva in children. *J Dent Res*. 1997;76:1074.

27. Messer LB. Assessing caries risk in children. *Aus Dent J*. 2000;45(1):10-16.
28. Navazesh M and Christensen CM. A comparison of whole mouth resting and stimulate salivary measurement procedures. *J Dent Res*. 1982;61:1158-1162.

29. Nevin RB and Walsh JP. Some physicochemical factors in relation to causation of interproximal caries. *J Dent Res*. 1951;30:235-250.

30. Office of Head Start, Income guidelines for participation in Head Start programs [online]. U.S. Department of Health & Human Services, 2006. Available from: http://www.acf.hhs.gov/programs/hsb/about/incomeguidelines/index.htm [Accessed in 17 Oct 2006]

31. Pinkham JR, Casamassimo PS, Fields HW, McTtigue DJ, and Nowak AJ. *Pediatric dentistry: infancy through adolescence,* 4th ed. St Louis: Elsevier Saunders, 2005. pp. 213-214 and 283-286.

32. Smith P M. Mechanisms of secretion by salivary glands. In: Edgar WM and O'Mullane DM, eds., *Saliva and oral health*, 2nd ed. London: British Dental Journal, Thanet Press Limited, Margate, 1990. pp. 9-25.

33. Soderling E. Practical aspects of salivary analysis. In: Tenovuo J, ed., *Human saliva: clinical chemistry and microbiology* vol. I, Boca Raton: CRC Press, 1989. pp. 1-24.

34. Sreebny LM., Xerostomia: Diagnosis, Management and Clinical Complications. In: Edgar WM and O'Mullane DM, eds., *Saliva and oral health*, 2nd ed. London: British Dental Journal, Thanet Press Limited, Margate, 1990. pp. 46-65.

35. ten Cate B. The role of saliva in mineral equilibria - Caries and calculus formation. In: Edgar WM and O'Mullane DM, eds., *Saliva and oral health*, 2nd ed. London: British Dental Journal, Thanet Press Limited, Margate, 1990. pp. 123-136.

36. Tinanoff N, and Douglass J. Clinical decision making for caries management in primary teeth. *J Dent Edu*. 2001;65(10):1133-1142.

37. Watanabe S. Salivary clearance from different regions of the mouths in children. *Caries Res*. 1992;26:423-427.

38. Watanabe S, Dawes C. Salivary flow rates and salivary film thickness in five-year-old children. *J Dent Res*. 1990;69:1150-1153.

39. Watanabe S, Ohnishi K, Imai E, Kawano E, Igarashi S. Estimation of the total saliva volume produced per day in five year old children. *Arch Oral Biol.* 1995;40:781-2.

40. Whelton H. The anatomy and physiology of the salivary glands. In: Edgar WM and O'Mullane DM, eds., *Saliva and oral health*, 2nd ed. London: British Dental Journal, Thanet Press Limited, Margate, 1990. pp. 1-8.

APPENDIX A

SALIVA STUDY INVITATION LETTER IN ENGLISH AND SPANISH

LOMA LINDA UNIVERSITY

School of Dentistry
Department of Pediatric Dentistry

Loma Linda, California 92350
(909) 558-4689
FAX: (909) 558-0322

Dear Parents,

We are writing you to invite your child to participate in a research project conducted by the Department of Pediatric Dentistry at Loma Linda University School of Dentistry. The objective of this study is to investigate saliva characteristics in three to four year old children with and without visible tooth decay. Your family's information will be kept anonymous and your child's name will not be used. Each child participating will be assigned a random study number in the collection and analysis of data.

Attached, you will find three forms:

1) An Informed Consent document which fully explains how the study will be conducted, the risks involved, and a confidentially agreement.
2) A questionnaire about your child's medical and dental history.
3) A questionnaire about family dietary and dental history.

By signing the enclosed Informed Consent document, you are indicating that you have discussed this with your child and are giving permission for your child's participation based on their willingness and ability to participate. Taking part in this research study is strictly voluntary and not part of Head Start. There is no obligation for your child to participate. Also, if you agree and then decide to change your mind, you can stop your child's participation at any time. If you have any questions about this research study please contact Dr. John E. Peterson or Dr. Gloria Yañez at (909) 558-4689.

Thank you.

Loma Linda University
Adventist Health Sciences Center
Institutional Review Board
Approved 12/7/06 **Void after** 12/6/2007
56303 **Chair** R L Rigsby MD

Estimados Padres,

Le estamos escribiendo para invitar a su niño(a) a participar en un estudio de investigación conducido por el Departamento de Odontología Pediátrica de la Escuela de Odontología de Loma Linda Universidad. El objetivo de este estudio es investigar las características de saliva de niños entre tres y cuatro años de edad cuando tienen caries o cuando no presentan caries visualmente. La información de su familia será mantenida anónima y el nombre de su hijo(a) no será utilizados. Cada niño(a) participando será asignado un número al azar en la coleción y el análisis de los datos.

Anexo a esta carta, encontrará tres formularios:

1) Un documento de consentimiento que explica como el estudio será conducido, los riesgos, y la confiencialidad del acuerdo.
2) Un cuestionario sobre la historia médica y dental de su niño(a).
3) Un cuestionario sobre la historia dietética de su niño(a) y la historia dental de la familia.

Si usted firma esta autorización, usted otorga su permiso y indica que hablo con su hijo(a) y tiene el deseo de participar en el estudio. Usted y su familia no estan obligados a participar. La participación en este estudio es voluntario y no es parte de Head Start. Usted también tiene el derecho a revocar la participación de su niño(a) en cualquier momento por cualquier motivo. Si tiene alguna duda sobre el estudio, Por favor llame al (909)558-4689 y hable con el Dr. John E. Peterson o la Dra. Gloria Yañez.

Muchas Gracias.

APPENDIX B

INFORMED CONSENT FORM IN ENGLISH AND SPANISH

LOMA LINDA UNIVERSITY

School of Dentistry
Department of Pediatric Dentistry

Loma Linda, California 92350
(909) 558-4689
FAX: (909) 558-0322

Informed Consent

"Saliva Characteristics in 3 to 4 year old Head Start Children With and Without Visible Caries"

PRINCIPAL INVESTIGATOR — John E. Peterson, DDS

CO-INVESTIGATOR — Gloria Yañez, DDS

Purpose: Your permission is requested to allow us to invite your child to participate in a study to investigate saliva characteristics in 3 to 4 year old children with and without visible tooth decay or cavities.

Procedures: If you give your permission for your child to participate, please sign this consent form and complete the two attached questionnaires about your family's medical, dental, and dietary history. An additional copy of the consent form will be provided for your records. Please return the completed forms to the Head Start teacher. Your child will not be asked to participate in the study unless the forms have been returned. Your child must show an interest and willingness to participate. If your child says "no" or is afraid then your child will not be included in the study. This study will be part of one of your child's morning classes.

If your child is willing to participate and is selected based on the saliva study requirements, then he/she will be screened for tooth decay or cavities. This is only a dental screening and not a comprehensive dental exam. No x-rays will be taken. In addition, your child must be able to demonstrate the ability to spit and chew gum. The children who meet the saliva study requirements will be assigned to one of the two study groups. Group I will consist of children with no visible evidence of tooth decay or cavities. Group II will consist of children with 3 or more visible cavities. Only cavities that can be seen will be allowed in the tooth decay group. However, there may be cavities present in each group that can not be seen. It is recommended that all children see their dentist on a regular basis for a comprehensive dental exam.

All dental screenings and saliva collections will take place on the school premises in the Loma Linda University Mobile Dental Van or in the Head Start classroom, depending on availability of the Mobile Dental Van. On the assigned days of saliva collection, the Head Start teacher will be instructed not to allow your child to eat or drink anything for at least 1 hour before the saliva sample is collected. Each child's participation will be for approximately 10 minutes to collect saliva samples. Photographs may be taken to illustrate how the study is done and will be used for school purposes only.

Initial: _____
Date: _____

"Saliva Characteristics in 3 year to 4 year old Head Start Children With and Without Visible Caries"

Two saliva samples will be collected. Initially, an investigator will demonstrate how the procedure will be done before a small group of children. Then each child will be shown individually while they sit comfortably in a chair. The first sample will be collected by spitting or draining saliva through a straw into a small container for 2 minutes. The second saliva sample will collected after they chew a small piece of unflavored paraffin wax for 1 minute and then spit their saliva into a small container for 2 minutes as they continue to chew the wax.

Risks: There is a minimal potential choking hazard in chewing the paraffin wax.

Benefits: You and your child are unlikely to benefit directly from participating in this study. The information learned from this study may help clinicians to determine if saliva testing is a good indicator of tooth decay in young children who have no visible cavities but are at high risk of getting tooth decay.

Confidentiality: Any information you provide in this study will be confidential and will be protected. Any published document or photographs resulting from this study will not disclose your child's identity without your permission. Each child will be assigned a random study number; no names will be used in the analysis of the data.

Reimbursement: Aside from a toothbrush and dental floss, no other compensation will be provided for participation in this study. Participation is strictly voluntary.

Impartial Third Party Contact: If you wish to contact an impartial third party not associated with this study, please contact the Office of Patient Relations, Loma Linda Medical Center, Loma Linda, CA 92350, at (909) 558-4647 for information and assistance.

Subject Rights: By signing this Informed Consent document, you are indicating that you have discussed this with your child and are giving permission for your child to participation based on their willingness and ability to participate. Taking part in this research study is strictly voluntary and not part of Head Start. There is no obligation for your child to participate. Also, if you agree and then decide to change your mind, you can stop your child's participation at any time.

Informed Consent Statement: "I have read the contents of this consent form and hereby give written voluntary consent for my child to participate in this study. By signing this consent form, it does not waive my or my child's rights, nor does it release the investigators, institution, or sponsors from their responsibilities. If my child is willing to participate and has given verbal consent then he/she can participate in this study given by the Loma Linda Pediatric Dental Clinic at the Head Start Center. All my questions concerning this study have been answered to my satisfaction and I have been given a copy of this consent form. If I have any questions or concerns I can call Dr. John E. Peterson or Dr. Gloria Yañez at (909) 558-4689."

Name of Child: _____ **Date:** _____

Signature of Parent or Guardian: _____

Initial: ____
Date: _____

Consentimiento para Participar en un Estudio de Investigacíon

"Características de Saliva en niños de Head Start entre 3 a 4 años de edad Cuando Tienen Caries o Cuando No Presentan Caries Visualmente"

INVESTIGADOR PRINCIPAL — John E. Peterson, DDS

CO-INVESTIGADOR — Gloria Yañez, DDS

Propósito: Solicitamos su permiso para que su niño(a) participe en un estudio de investigación características salivares en niños entre 3 a 4 años de edad cuando tienen caries o cuando no presentan caries visualmente.

Procedimientos: Si usted da su permiso, por favor firme este documento de consentimiento y complete los dos cuestionarios sobre la historia médica, dental, y dietética de su familia. Se le entregará una copia de este formulaio de autorización/consentimiento para sus documentos. Por favor, devolve estas formularios a la maestra de Head Start. Incluso si usted otorga su autorización, su hijo(a) puede decider no participar, especialemente si no quiere cooperar o tiene miedo participar, no será incluyido en el estudio. Su hijo(a) debe de demostrar interés y el deseo de participar. Este estudio será parte de una clase en la mañana.

Si su hijo(a) está dispuesto a participar y resuelve los requisitos del estudio, examinaran sus dientes para determinar si tiene o no tiene caries visibles. Este examen es sencio y no se tomará ningunas radiografías para determinar caries. Sin embargo, su hijo(a) puede tener caries que no se ven y solamente pueden ser determinadas con radiografías. Además, su hijo(a) debe demostrar la capacidad de escupir y masticar goma. Asignarán los niños seleccionados a uno de dos grupos. Grupo I consistirá de niños y niñas sin la evidencia visible de caries. Grupo II consistirá de niños y niñas con tres o más caries que son visibles. Es muy importante que sus niños visiten el Dentista regularmente y obtenga un examen comprensivo.

Todas las investigaciones y colecciones de saliva ocurrirán en el local de la escuela de Head Start en la furgoneta dental móvil de la Universidad de Loma Linda o en la sala de clase, dependiendo de la disponibilidad de la furgoneta dental. En los días asignados para coleccionar saliva, es necesario no permitir que su hijo(a) come o bebe cualquier cosa por lo menos de una hora antes de colectar la muestra de saliva. La participación de cada niño(a) será por aproximadamente 10 minutos para recoger muestras de saliva. También es posible que tomaran fotos para documentar el estudio solamente para educar y servir el caso de la escuela.

Antes de obtener dos muestras de saliva, un investigador presentara el procedimiento antes de un grupo de niños. Despues, una explicación por cada niño(a) individualmente mientras que esten sentados comfortablemente en una silla. La primera muestra recogida es la saliva que escupen o se desagüe por un popote en un envase pequeño por 2 minutos. La segunda muestra es la saliva recogida despues de masticar un pequeño pedazo de cera parafina por un minuto. Entonces escupirán su saliva en un envase pequeño por 2 minutos mientras que continúan masticando la cera de parafina.

Iniciales: _____
Fecha: _____

"Características de Saliva en niños de Head Start entre 3 a 4 años de edad Cuando Tienen Caries o Cuando No Presentan Caries Visualmente"

Riesgos: Hay un peligro potencial minimo de ahogarse masticando el pedaso de cera parafina.

Beneficios: Usted y su hijo(a) no se benefician personalmente participando en este estudio de investigación. Esta información puede ayudar los clínicos a determinar si la prueba de saliva es un buen indicador de la susceptibilidad de caries en los niños que tienen el riesgo elevado.

Confidencialidad: Le aseguramos privacidad y confidencialidad de su información y será protegida. Ningún documento o fotografías publicado que resultan de este estudio divulgará la identidad de su hijo(a) sin su permiso. Asignarán cada niño(a) un número al azar; no se utilizará ningunos nombres en el análisis de los datos.

Compensación: Aparte de un cepillo y seda dental para su hijo(a), no habra ninguna otra remuneración por la participación en este estudio. Su decisión de participar es completamente voluntaria.

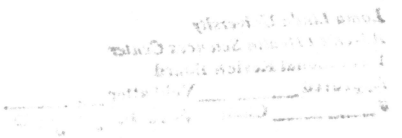
Contactos Imparciales: Si usted desea y tiene preguntas o inquietudes acerca de sus derechos puede ponerse en contacto con la Oficina de Relaciones de Pacientes, Loma Linda Medical Center, Loma Linda, CA 92350, (909) 558-4647 para información y ayuda.

Los Derechos del Participante: Si usted firma esta autorización, usted otorga su permiso y indica que hablo con su hijo(a) y tiene el deseo de participar en el estudio. Usted y su familia no estan obligados a participar. La participación en este estudio es voluntario y no es parte de Head Start. Usted también tiene el derecho a revocar la participación de su niño(a) en cualquier momento por caulquier motivo.

Acuerdo de Participación y Autorización/Consentimiento: "He leído el contenido de este formulario de Autorización/Consentimiento y doy mi permiso y otorga para que mi hijo(a) participe en este studio de saliva. Firmando este formulario de Autorización/Consentimiento, no renuncio mis derechos ni los de mi niño(a), ni desprend los investigadores, la institución, o los patrocinadores de sus responsabilidades. Si mi hijo(a) está dispuesto a participar y ha dado su consentimiento verbal entonces puede participar en este estudio dado por la clínica dental pediátrica de Loma Linda en el centro de Head Start. Todas mis preguntas referentes a este estudio se han contestado a mi satisfacción y me han dado una copia de este formulario de Autorización/Consentimiento para mi información. Si tengo cualquiera pregunta o preocupaciones puedo llamar al (909) 558-4689 y hablar con el Dr. John E. Peterson o la Dra. Gloria Yañez."

Nombre del niño(a): _____ Fecha: _____
Firma del pariente o del guardián: _____

Iniciales: ____
Fecha: _____



APPENDIX C

MEDICAL AND DENTAL HISTORY QUESTIONNAIRE IN ENGLISH AND SPANISH

Medical and Dental History

Child's Information

First Name _____ Last Name _____

Date of Birth _____ Age _____ Sex _____

General Health Information

Please answer each of the following questions. Check Yes or No. If in doubt, leave blank.

1. Does your child have any health problems? Yes No
   If yes, please explain: _____
2. Has your child had any physical or mental problems since birth? Yes No
   If yes, please explain: _____
3. Is your child taking any medicines or drugs at this time? Yes No
   If yes, please explain: _____
4. Does your child have any history of allergic reactions to any medicines or foods? Yes No
   If yes, please explain: _____
5. Does your child have any history of allergic reactions to any materials (Latex)? Yes No
   If yes, please explain: _____
6. Has your child ever been hospitalized or seriously injured? Yes No
   If yes, please explain: _____
7. Has your child had any history of the following?
   - ☐ Seasonal Allergies
   - ☐ Asthma
   - ☐ Heart Trouble or Murmur
   - ☐ Congenital Heart Disease
   - ☐ Hepatitis
   - ☐ Hemophilia or Bleeding Problems
   - ☐ Blood Disorders
   - ☐ Blood Transfusions
   - ☐ Growth Problems
   - ☐ Neurological Problems
   - ☐ Mental or Emotional Problems
   - ☐ Diabetes
   - ☐ Kidney/Liver Problems
   - ☐ Breathing or Lung Problems
   - ☐ Rheumatic Fever
   - ☐ Sickle Cell Anemia
   - ☐ Cancer/Chemotherapy
   - ☐ Seizures/Epilepsy/Convulsions
   - ☐ Loss of Consciousness/Fainting
   - ☐ Other _____
8. Does your child have any problems with:
   ☐ Concentration ☐ Cooperation ☐ Understanding ☐ Speech
9. Do you think your child will be interested and cooperative for the saliva study? Yes No

General Dental Information

1. Has your child ever seen a Dentist? Yes No
   If yes, please explain: _____
2. Has your child ever had or has any of the following?
   - ☐ Toothaches/Facial Pain
   - ☐ Dental Infection/Abscess
   - ☐ Bleeding Gums
   - ☐ Bad Breath
   - ☐ Dental Caries/Cavities
   - ☐ Cold Sores/Fever Blisters
   - ☐ Stained Teeth
   - ☐ Trauma/Injuries to Teeth
3. Does your child have any habits which might affect their oral health?
   - ☐ Sleeps with a Baby Bottle
   - ☐ Pacifier
   - ☐ Finger or Thumb Habits
   - ☐ Mouth Breathing
   - ☐ Tongue Habits
   - ☐ Grinds Teeth
4. What is your child's Fluoride source?
   - ☐ Water
   - ☐ Fluoride Toothpaste
   - ☐ No Fluoride
   - ☐ Fluoride Tablets
   - ☐ Fluoride Rinses

My signature indicates that I have answered all questions on the medical and dental health history to the best of my knowledge. This information will be kept confidential and will only be used for the study purposes. If you have any questions or concerns, please contact Dr. Gloria Yañez, or Dr. John E. Peterson at (909) 558-4689.

Parent or Guardian Signature _____ Date _____

Historia Medico y Dental

Información del Niño(a)

Nombre _____ Apellido _____

Fecha de Nacimiento _____ Edad _____ Sexo _____

Información de la Salud General

Por favor conteste las siguientes preguntas. Marque Sí o No. Si hay duda, no lo marque.

1. ¿ Ha tienido o tiene su hijo(a) algún problema de salud? Sí No
   Si es afirmativo, explíque: _____
2. ¿Tuvo su hijo(a) algún problema de salud al nacer? Sí No
   Si es afirmativo, explíque: _____
3. ¿ Esta tomando o recibiendo su hijo(a) alguna medicina o droga actualmente? Sí No
   Si es afirmativo, explíque: _____
4. ¿ Ha tenido su hijo(a) alguna reaccíon o alergia a la comida o medicina? Sí No
   Si es afirmativo, explíque: _____
5. ¿ Ha tenido su hijo(a) alguna reaccíon o alergia a materiales o otras cosas? (ej. Latex) Sí No
   Si es afirmativo, explíque: _____
6. ¿ Ha estado su hijo(a) hospitalizado, o se ha golpeado en un accidente gravemente? Sí No
   Si es afirmativo, explíque: _____
7. ¿ Ha tenido su hijo(a) alguna de las siguiente condiciones o enfermedades?
   - ☐ Alergias Temporales /Sinusitis
   - ☐ Asma
   - ☐ Enfermeda de Corazón o Soplo
   - ☐ Diabetes
   - ☐ Hepatitis
   - ☐ Hemofilia o Problemas de Sangramiento
   - ☐ Enfermeda de la Sangre
   - ☐ Transfusiones de Sangre
   - ☐ Problemas de Crecimiento
   - ☐ Problemas de Neurológicos
   - ☐ Problemas Mentales o Emocionales
   - ☐ Enfermedas de Respiración o de pulmones
   - ☐ Enfermedas de Riñónes o Hígado
   - ☐ Cáncer/Quimoterapia
   - ☐ Fiebre Reumática
   - ☐ Anemia Falsiforme
   - ☐ Epilepsia/Convulsiones/Apoplejía
   - ☐ Desmayos
   - ☐ Otra _____
8. ¿ Tiene su hijo(a) problema con alguna de las siguiente condiciones?
   ☐ Concentración ☐ Cooperación ☐ Entiendimiento ☐ Habla
9. ¿ Usted piensa que su hijo(a) estar interesada y copera en el estudio de saliva? Sí No

Información de la Salud Dental

1. ¿ Ha visitado su hijo(a) el Dentista? Sí No
   Si es afirmativo, explíque: _____
2. ¿ Ha tenido o tiene su hijo(a) alguna de las siguientes condiciones?
   - ☐ Dolor de Diente o Muela/ Dolor en la Cara
   - ☐ Infección Dental /Absceso
   - ☐ Sangramiento de Encías
   - ☐ Mal Aliento
   - ☐ Caries Dentales
   - ☐ Ulceras/Infecciónes en los labios
   - ☐ Dientes Manchados
   - ☐ Golpes o Trauma/Fracturas de Dientes
3. ¿ Ha tenido o tiene su hijo(a) alguna hábitos que puede afectar su salud oral?
   - ☐ Duerme con el biberón
   - ☐ Chupón
   - ☐ Chupeteo de dedo
   - ☐ Respira por la boca
   - ☐ Hábitos con la lengua
   - ☐ Rechina los dientes
4. Recibe Fluoruro por?
   - ☐ Agua
   - ☐ Pasta dental con Fluoruro
   - ☐ Sin Fluoruro
   - ☐ Tabletas de Fluoruro
   - ☐ Enjuagues de Fluoruro

Al firmar este papel certifico que he comprendido y contestado cada pregunta del historial medico y dental de mi hijo(a) a lo mejor que yo sé. Esta información es confidencial y va ser usada solomente en este estudio de saliva. Si usted tiene alguna pregunta o una duda, puede llamar a la Dra. Gloria Yañez, o el Dr. John E. Peterson al (909) 558-4689.

Firma del Padres/ Guardián _____ Date _____

APPENDIX D

MEDICAL AND DENTAL HISTORY QUESTIONNAIRE IN ENGLISH AND SPANISH

Family Dietary and Dental History Questionnaire

Child's Name: _____ **Date:** _____

Parents or Guardians please respond to each of the dietary questions as if for a normal day at home.

Dietary

1. How many times a day does your child eat sugary snacks (e.g. candy, cookies, pastries, chips, etc) between meals or instead of meals? _____
2. How many times a day does your child drink sugar drinks (e.g. regular sodas, Kool-aid, lemonade, fruit juices, ice tea, etc) instead of water? _____
3. How many times a day does your child drink water (not flavored water)? _____
4. How often does your child fall asleep with a baby bottle or sippy cup containing liquids other than water? _____

Total _____

Dental

1. What age was your child's first visit to the dentist? _____
2. Does your family have a dentist that you or your family sees on a regular basis? Yes No
3. Mom or Immediate Caregiver, do you have any cavities or tooth decay? Yes No
4. Does anyone else in your family have any cavities or tooth decay? Yes No
5. Do you help your child brush their teeth regularly? Yes No
6. Does your child brush their teeth alone? Yes No
7. Do you use dental floss to clean your child's teeth? Yes No

Cuestionario de Historia Dental y Dietética Familiar

Nombre del niño(a): _____ **Fecha:** _____ **Número:** _____

Por favor Padres o Guardianes responden a cada pregunta del dietético como si fuera para un día normal en su casa.

Dietético

1. ¿Cuántas veces al día come su niño(a) bocados (por ejemplo: dulces, galletas, pasteles, papas fritas, etc) entre o en vez de comidas? _____
2. ¿ Cuántas veces al día toma su niño(a) bebidas con azúcar (por ejemplo: Kool-aid, sodas, limonadas, jugos, té, etc) en vez de agua? _____
3. ¿ Cuántas veces al día toma su niño(a) agua? _____
4. ¿ Cuántas veces se duerme su niño(a) con el biberon o un vasito de Bebé que contenga leche o otros líquidos menos agua? _____

Total _____

Dental

1. ¿A qué edad de su niño(a) fue la primera visita al dentista? _____
2. ¿Tiene su familia un Dentista que vistan regularmente? Sí No
3. ¿Mamá o Guardian, tiene usted caries o dientes picados? Sí No
4. ¿Hay alguien más en su familia que tiene los dientes picados? Sí No
5. ¿ Ayuda usted a su niño(a) a cepillar sus dientes regularmente? Sí No
6. ¿ Se cepilla su niño(a) sus dientes solo(a)? Sí No
7. ¿ Utiliza usted el hilo dental para limpar los dientes de su niño(a)? Sí No

APPENDIX E

AAPD CARIES-RISK ASSESSMENT TOOL (CAT)[2]

AAPD Caries-Risk Assessment Tool (CAT)

RISK FACTORS TO CONSIDER (For each item below, circle the most accurate response found to the right under "Risk Indicators")	RISK INDICATORS High	Moderate	Low
Part 1- History (determined by interviewing the parent/primary caregiver)			
Child has special health care needs, especially any that impact motor coordination or cooperation[A]	Yes		No
Child has condition that impairs saliva (dry mouth)[B]	Yes		No
Child's use of dental home (frequency of routine dental visits)	None	Irregular	Regular
Child has decay	Yes		No
Time lapsed since child's last cavity	<12 months	12 to 24 months	>24 months
Child wears braces or orthodontic/oral appliances[C]	Yes		No
Child's parent and/or sibling(s) have decay	Yes		No
Socioeconomic status of child's parent[D]	Low	Mid-level	High
Daily between-meal exposures to sugars/cavity-producing foods (includes on demand use of bottle/sippy cup containing liquid other than water; consumption of juice, carbonated beverages, or sports drinks; use of sweetened medications)[E]	>3	1 to 2	Mealtime only
Child's exposure to fluoride[F,G]	Does not use fluoridated toothpaste; drinking water is not fluoridated and is not taking fluoride supplements	Uses fluoridated toothpaste; usually does not drink fluoridated water and does not take fluoride supplements	Uses fluoridated toothpaste; drinks fluoridated water or takes fluoride supplements
Times per day that child's teeth/gums are brushed	<1	1	2-3
Part 2 - Clinical evaluation (determined by examining the child's mouth)			
Visible plaque (white, sticky buildup)	Present		Absent
Gingivitis (red, puffy gums)[H]	Present		Absent
Areas of enamel demineralization (chalky white-spots on teeth)	More than 1	1	None
Enamel defects, deep pits/fissures[I]	Present		Absent
Part 3- Supplemental professional assessment (Optional)[J]			
Radiographic enamel caries	Present		Absent
Levels of mutans streptococci or lactobacilli	High	Moderate	Low

Each child's overall assessed risk for developing decay is based on the highest level of risk indicator circled above (ie, a single risk indicator in any area of the "high risk" category classifies a child as being "high risk").

A. Children with special health care needs are those who have a physical, developmental, mental, sensory, behavioral, cognitive, or emotional impairment or limiting condition that requires medical management, health care intervention, and/or use of specialized services. The condition may be developmental or acquired and may cause limitations in performing daily self-maintenance activities or substantial limitations in a major life activity. Health care for special needs patients is beyond that considered routine and requires specialized knowledge, increased awareness and attention, and accommodation.

B. Alteration in salivary flow can be the result of congenital or acquired conditions, surgery, radiation, medication, or agerelated changes in salivary function. Any condition, treatment, or process know or reported to alter saliva flow should be considered an indication of risk unless proven otherwise.

C. Orthodontic appliances include both fixed and removable appliances, space maintainers, and other devices that remain in the mouth continuously or for prolonged time intervals and which may trap food and plaque, prevent oral hygiene, compromise access of tooth surfaces to fluoride, or otherwise create an environment supporting caries initiation.

D. National surveys have demonstrated that children in low-income and moderate-income households are more likely to have caries and more decayed or filled primary teeth than children from more affluent households. Also, within income levels, minority children are more likely to have caries. Thus, socioeconomic status should be viewed as an initial indicator of risk that may be offset by the absence of other risk indicators.

E. Examples of sources of simple sugars include carbonated beverages, cookies, cake, candy, cereal, potato chips, French fries, corn chips, pretzels, breads, juices, and fruits. Clinicians using caries-risk assessment should investigate individual exposures to sugars known to be involved in caries initiation.

F. Optimal systemic and topical fluoride exposure is based on use of a fluoride dentifrice and American Dental Association/American Academy of Pediatrics guidelines for exposure from fluoride drinking water and or supplementation.

G. Unsupervised use of toothpaste and at-home topical fluoride products are not recommended for children unable to expectorate predictably.

H. Although microbial organisms responsible for gingivitis may be different than those primarily implicated in caries, the presence of gingivitis in an indicator of poor or infrequent oral hygiene practices and has been associated with caries progression.

I. Tooth anatomy and hypoplastic defects (e.g., poorly formed enamel, developmental pits) may predispose a child to develop caries.

J. Advanced technologies such as radiographic assessment and microbiologic testing are not essential for using this tool.