A. 'Exploring the Relationship between Specific Salivary Components, Dental Caries and Aging'

Case Report 2 submitted in partial fulfillment of the Residency in Dental Public Health

By

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B. ABSTRACT: Objective: The purpose of this study is to explore the relationship between individual salivary components, dental caries and age, utilizing the data collected from the Oral Health: San Antonio Longitudinal Study of Aging (OH:SALSA). Methods: The study population comprised a well-defined stratified sample of 1148 Mexican American and European American men and women. Subjects were divided into six age groups from 35 to 75+ years old. Unstimulated/stimulated parotid and unstimulated/stimulated submandibular/sublingual saliva flow rates, total protein, 6 individual proteins and 4 inorganic constituents were measured. Specific salivary components were Lactoferrin, IgA, Albumin, Lysozyme, Mucin, Cystatin, Potassium, Calcium, Sodium and Chloride. Caries measurement was the DMFT Index for crowns and for roots, Tissue Health Index for crowns and for roots, Tooth caries, Root caries and Tooth restoration. The data was square root transformed for linearity prior to analysis. Analysis was carried out in two stages. Partial correlation was performed, in order to identify significant relationships between salivary flow rate, salivary components, and caries measurements, controlling for age group. Then in the second stage, using caries measurement as the dependant variable, several models were used to examine the effects of age, flow rate, concentration and output (product of flow rate and concentration). Using specific salivary components models were examined with flow rate, component concentration and output as independent variables. Results: In the function of these glands, significant associations were found between caries, age and specific individual proteins (Lactoferin, Albumin, Lysozyme, Mucin and Cystatin) and specific inorganic constituents (Potassium, Calcium, Sodium, and Chloride). The biologic understanding of these associations will be the subject of additional work.

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C. SPECIFIC ROLES:

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The specific roles played by me as researcher was in literature review, analyzing, interpreting and $\rho \not \mid \mathcal{D}$ reporting this study. I was assisted by Stephanie Cano MS (Consulting statistician), Dr Howard Dang PhD (Consultant), Dorthea Alvares Johnson MS (Consultant).

D. STATEMENT OF PURPOSE:

The purpose of this study was to explore the relationship between individual salivary components and dental caries, utilizing the data collected from the Oral Health: San Antonio Longitudinal Study of Aging (**OH:SALSA**). Purpose of the **SALSA** study was to test a cohesive set of hypotheses designed to enhance our understanding of: 1) ethnic differences in functional status and the burden of disease in the elderly Mexican Americans (MAs) and non-Hispanic Whites, 2) Socio-cultural determinants of functional status and the burden of disease in the elderly MAs populations, and 3) the association between functional status and the burden of disease, with focus on the functional sequelae of four major chronic diseases: non-insulin dependant diabetes mellitus (NIDDM) and its complications, coronary heart disease, hypertension and arthritis. The oral health component (OH: SALSA) was later included and measured as a part of this study.

E. BACKGROUND AND REVIEW OF LITERATURE:

Saliva plays an essential role in the maintenance of oral health. A reduction in salivary gland function may result in dental caries, difficulties in swallowing, speech, denture wearing problems, alteration in taste, and increased frequency of opportunistic infections (1).

Major salivary glands: The major salivary glands include the paired **parotid**, **submandibular** and **sublingual** glands. The parotid is the largest salivary gland and saliva is secreted into the mouth via the parotid duct (Stensen's duct). The submandibular gland lies inferior to the body of

the mandible. Its drainage is via the duct of the submandibular gland (Wharton's duct) into the floor of the mouth on either side of the lingual frenulum. The sublingual glands are situated under the mucosa in the floor of the mouth, on the sides of the tongue (2).

Minor salivary gland: The minor salivary glands lack a branching network of draining ducts. Instead, each salivary unit has its own simple duct. In addition, 600-1,000 minor salivary glands line the oral cavity and oropharynx, contributing only a small portion of total salivary production (3, 4).

Composition of Saliva: In general, saliva is composed of 99.5% water. It is also composed of a variety of electrolyte, including sodium, potassium, calcium, magnesium, bicarbonate, and phosphates. Also found in saliva are immunoglobulins, proteins, enzymes, mucins, and nitrogenous products, such as urea and ammonia. The normal pH of saliva is between 6-7 (5).

Salivary Flow: The average volume of saliva secreted in a 24 hour period is 1-1.5 liters (approximately 1 cc/minute) most of which is secreted during meals. The basal salivary flow rate is 0.001-0.2 ml/minute/gland. With stimulation, salivary flow rate ranges 0.18-1.7 ml/min/gland. Salivary flow rate from the minor salivary glands is independent of stimulation, constituting 7-8% of total salivary output (3). Salivary flow rate exhibits both diurnal and seasonal variations which peaks in mid afternoon and higher flow rates in the spring than autumn. During sleep the flow rate is negligible (1). In the **unstimulated** state the relative contribution of the major salivary glands is as follows: 1) Submandibular gland - 69%, 2) Parotid gland - 26%, 3) Sublingual gland - 5%. In the **stimulated** state the relative contribution of the major salivary glands is as follows: 1) Parotid gland - 69%, 2) Submandibular gland - 26%, 3) Sublingual gland - 5%. Though the Sublingual glands and minor salivary glands contribute only about 10% of all saliva, together they produce the majority of mucous and are critical in maintaining the mucin

layer over the oral mucosa (1).

Functions of Saliva (Table 1): The complexity of this oral fluid is perhaps best appreciated by the consideration of its many and varied functions. The functions of saliva are largely protective. Some important functions of saliva include 1) lubricating the oral tissues to assist with swallowing and speaking 2) buffering cariogenic acid 3) participating in forming the dental pellicle 4) serving as a supersaturated mineral source for the hard tissues and 5) protecting the oral tissues against microbial infections (6).

Saliva and dental caries: Saliva is well adapted to protection against dental caries. Saliva's buffering capability; the ability of the saliva to wash the tooth surface, to clear bacteria, and to control demineralization and mineralization, saliva's antibacterial activities, and perhaps other mechanisms all contribute to its essential role in the health of teeth. The fact that the protective function of saliva can be overwhelmed by bacterial action indicates the importance of prevention and therapy as in other infectious diseases. A more complete knowledge of functional properties of saliva as well as those of its separate components may permit a better assessment of dental caries susceptibility. Dental caries also depends on the influence of independent risk factors that interact with the salivary components in a protective, as well as in a risk-increasing manner. These independent risk factors are saliva, fluoride, oral hygiene, diet and time.

Salivary components: Individual components of saliva have been shown to affect either bacterial activity or demineralization/remineralization of the tooth structure. Some salivary substances have direct bactericidal or bacteriostatic effects; others can cause aggregation of oral bacteria resulting in an increased clearance of oral bacteria. Still others affect more directly the physical properties of saliva and the tooth.

Lactoferrin: Lactoferrin is an iron-binding protein with certain similarities to transferrin, the

iron-binding protein found in the blood. Lactoferrin has been shown to have antimicrobial activity. To display this activity in the oral cavity; lactoferrin binds to two iron atoms per molecule and in so doing prevents iron from being used by organisms that require it for metabolism. Organisms most susceptible are anaerobic and facultative anaerobic bacteria. In addition, lactoferrin appears to have an antimicrobial activity that is independent of its ability to bind iron (7). Growth of streptococcus mutans is sensitive to lactoferrin, and the inhibition appears to be iron independent (8)

Lysozyme: Lysozyme is an enzymatic protein that has direct antimicrobial effects. It is positively charged and binds to salivary anions of various types, including bicarbonate, fluoride, iodine, and nitrate. When combined with these anions the complex binds to the cell wall of bacteria and destabilizes the wall by catalyzing the hydrolysis of glycosidic bonds in the polysaccharides component of the wall and allowing autolysis to take place (9). The antimicrobial effect has been shown to be exerted against mutans streptococci. The enzyme also appears to alter intermediary glucose metabolism in sensitive bacteria and in some cases, causes aggregation, perhaps contributing to clearance of bacteria from the oral cavity. Its ability to bind to hydroxyappatite suggests an antimicrobial role on the tooth surface.

Cystatins: Cystatins are a group of cystein-enriched protease inhibitors with an average mass of about 15 kD. As protease inhibitors, they prevent the action of potentially harmful proteases on the soft tissue of the oral cavity. The cystatins also bind to hydroxyappatite, however, this acidic cystein-containing protein inhibits precipitation of calcium phosphate and protects the tooth surface by promoting supersaturation of saliva with calcium and phosphate (10, 11).

Mucins: Mucins play a multiple role in the oral cavity. The major salivary mucins are MG1 and MG2. MG1 absorbs tightly to the tooth surface. It has a primary role of contributing to the

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enamel pellicle thereby protecting the tooth surface from chemical and physical attack, including acid challenges. Human MG2 is the better characterized of the mucins. It can also bind to the tooth surface but is easily displaced. More importantly, by aggregation it promotes clearance of oral bacteria, including mutans streptococci, from the oral cavity. The ability to cause aggregation has been reported to be directly related to promotion of caries resistance. Aggregating activity seems to reside on the carbohydrate portion of the MG2, much of it residing on a specific trisaccharide. The ability of certain bacteria to catalyze the hydrolysis and removal of these sugar moieties and the ability of the bacteria to remove sulphur from the molecule may reduce the ability of MG2 to act as a clearance factor for those bacteria (12, 13). The ability to aggregate bacteria appears to be shared by a number of glycoproteins present in human saliva (14, 15, and 16). These glycoproteins can be involved both in adherence of bacteria to the surface and in clearance from the oral cavity. Mucin composition and the degree of its proteolysis in the oral cavity may be related to caries status (17). Bacterial binding selectivity is a property of these proteins that need further study.

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Salivary IgA: IgA represents the principal immunoglobulin found in the saliva. The secretory component is added to the molecule by the secretory cells and acts as a part of the membrane receptor for IgA. The secretory component protects IgA from proteolytic attack (18). Secretory IgA is a defense protein inhibiting pathogens entering the gastrointestinal tract. Secretory IgA has also been shown to inhibit bacterial adherence to dental enamel depending on the strain of bacteria analyzed. Its presence in the salivary pellicle indicates that it is intimately related to the tooth surface. The ability of the secretory IgA to inhibit adherence appears to be related to its ability to bind to surface adhesions of the bacteria as well as to neutralize their negative surface charge. IgA has been shown to bind to mutans streptococci facilitating bacterial aggregation and

removal from the oral cavity. Secretory IgA molecules are multivalent antibodies and can prevent the adverse effects of bacterial toxins and enzymes. Their relative effect on tooth surface as a result of their binding to bacteria is not well defined and depends on several other factors. **Other proteins:** α amylase is not discussed, as it has little direct antibacterial effects, despite potential effects in making glucose available in plaque metabolism. The activity of oral bacteria bound to proline-rich proteins and their role in caries development remains an important issue. Saliva and aging: Aging can be separated into 2 different type's primary and secondary aging. The concept of primary aging hypothesizes that alterations of physiological function with advancing age are due to passage of time, and are independent of extrinsic physical and psychological disturbances such as stress, trauma and disease. Conversely, secondary aging implies that the functional status with increasing age is the result of external influences, including systemic disease, superimposed on chronological aging (19). It is well recognized that alterations of salivary gland function in the elderly are commonly associated with age related diseases and their therapeutic treatments (Secondary aging) (20). Flow rates of stimulated and unstimulated submandibular/subligual saliva usually show age related decreases in flow rates. The population shows no systematic change in parotid gland composition with age, (21). In the submandibular/sublingual gland the secretory IgA, acidic proline rich proteins and lysozyme show no change with age while chloride, calcium and histatins decrease. Amylase and sodium are either unchanged or reduced while potassium and lactoferrin are unchanged or increased with Total protein may be unchanged or it may increase or decrease. While age. submandibular/sublingual saliva comprises of the majority of the saliva in the mouth (22), the effects of aging on the composition of this glandular secretion received little attention. One report indicated a decrease in total protein secretory rate with increasing age (23). Another found

a decrease in histatin concentrations and secretory rates (24) with age.

F. STUDY DISCRIPTION

It has been shown from the OH:SALSA by Johnson DA that elderly individuals (> 65 yrs) have higher caries than younger groups (32-65 yrs) and a major factor for higher caries among this age group is the saliva flow rate, specially submandibular/sublingual saliva flow rate (25). Saliva is composed of many anticariogenic proteins (26, 27). The purpose of this study was to explore from the OH-SALSA data base the relationship between salivary anticariogenic proteins, controlled for flow rate, and dental caries in stratified age groups.

G. PROCEDURES AND METHODS

The aim of this study was to determine the relationship between present salivary components, past and present caries and aging.

Saliva collection: saliva was collected from all major salivary glands, and defined as follows: unstimulated and stimulated parotid saliva, unstimulated and stimulated submandibular and sublingual saliva. Flow rates were also determined. Details of the methods are to be found in earlier publication of OH:SALSA (24, 28, 29).

Analysis of the salivary components: The method for sodium, potassium, chloride, total protein, secretory IgA, lactoferrin, lysozyme, cystatin and albumin have been described previously (24, 28, 29). Calcium was determined by flame spectrophotometry. Mucin were quantitated using a polyacrlyamide gel separation technique (30), followed by staining with periodic acid Schiff reagent (Zacharis). Total protein was estimated by spectrophotometric absorbance at 215 nm using dilution of BSA as standards (31).

POPULATION AND SAMPLE (Table 2)

The population density in San Antonio is 1,084.4/km² (2,808.5/mi²). There are 433,122 housing

units at an average density of 410.3/km² (1,062.7/mi²) (32).

Selection of study population: Subjects were participants in the Research Center for Oral Health and Aging: 'Oral Health: San Antonio Longitudinal Study of Aging' (OH:SALSA). This cross sectional assessment was of oral health and functional status of individuals recruited from the San Antonio Longitudinal Study of Aging (SALSA). These are cross-cultural and community based studies, with populations comprised of a well-defined sample of Mexican American and European American men and women. Subjects were recruited from three socio-economically distinct San Antonio neighborhoods: low-income barrio, middle-income transitional and upper income suburban. The OH: SALSA participants also represent a stratified sample of the population's demographics in the greater San Antonio area.

Sample size: The total sample size of 1148 individuals was included in this current data analysis. **Study Design:** This study was a cross sectional survey design, where single assessments of oral health status of individuals were carried out, across a range of ages.

SURVEY PARAMETERS FOR OH: SALSA

The epidemiological assessment has 2 major oral health components: 1) a comprehensive questionnaire developed from NHANES III, and 2) a comprehensive clinical assessment of oral health status by a team of dentist examiners and recorders. Caries measurement was defined to include a variety of indices: Tissue Health Index for crown and for roots, DMFT Index for crowns and for roots, tooth crowns with caries (DT) and tooth crowns with restoration (FT).

Comprehensive clinical assessment of oral health: An extensive set of calibration procedures were developed by NIDR; these were used to train examiners and recorders in OH:SALSA. The data was entered by keyboard directly into a computer terminal at the examination site in the dental school. Opscan forms are provided, as a backup system for recording data should the

computer system failed to function properly. The computer display screen was patterned after the Opscan forms. Two calibrated dentists and two recorders from the Clinical Research Facility at UTHSCSA conducted the Oral Examination Section assessments. These assessments include: 1) Examination of full mouth oral mucosa. 2) Charting of restoration, caries, and periodontal status, 3) temporomandibular disorder examination, 4) esthetic evaluation, and 5) pulp test.

In **saliva collection**, two trained saliva collection personnel with two assistants measured salivary flow rates, collecting unstimulated whole and unstimulated and stimulated parotid, unstimulated and stimulated submanibular/sublingual saliva samples and cytological smears for the presence of candidal psuedohypae.

Modified Tissue Health Index for teeth: T-Health is an index intended to represent the total amount of an individual's sound tooth tissue at a particular point in time. The number of sound-equivalent teeth, is defined as a weighted average of sound teeth, filled (otherwise sound) teeth and teeth with some decay, the weights being, in principle, intended to represent the relative amounts of sound tissue in these three categories of teeth. The Tissue Health Index was modified for this study and varies from 0 (edentulous) to 1 (all teeth present and sound) (33, 34).

Formula used: THM_C = (# decayed crowns + # filled crowns + 4*# sound crowns)/ (4*28) THM R = (# decayed roots + # filled roots + 4*# sound roots)/ (4*28)

ETHICAL APPROVAL:

Informed consent was obtained from each participant in the OH:SALSA. The Institutional Review Board of the University of Texas Health Science Centre, San Antonio, approved SALSA study and the protocol.

STATISTICAL ANALYSIS

Specific salivary components were Potassium, Calcium, Sodium, Chloride, Lactoferrin, IgA,

Albumin, Lysozyme, Mucin and Cystatin. The data was square root transformed for linearity prior to analysis. Analysis was carried out in two stages. Partial correlation was performed, in order to identify significant relationships between salivary flow rate, salivary components, and caries measurements, controlling for age group. Then in the second stage, using caries measurement as the dependant variable, several models were used to examine the effects of age, flow rate, concentration and output (product of flow rate and concentration). Using specific salivary components as the dependant variable, models were examined with flow rate, component concentration and output as independent variables. The 3 models proposed are as follows: 1) Flow Rate Model: Independent variables are age group, flow rate, the interaction between age group and flow rate 2) Flow Rate Concentration Model: Independent variables were age group, flow rate, concentration, the interaction between age group and flow rate and concentration 3) Output Model: Independent variables were output and the interaction between age group and concentration 3) Output Model: Independent variables were output and the interaction between age group and output. The most appropriate model to explain caries with aging in relation to specific salivary constituents was selected and justified.

H. RESULTS

Flow rate Model (Table 3): This model tested flow rate and the age group by flow rate interaction term as covariates for the caries measures. Flow rate was observed to be a significant (p<0.001) covariate for DMFT and THM (Tissue Health Index) crown and root caries measures in both stimulated and unstimulated submandibular/sublingual saliva. When the DMFT-root caries measure was predicted by the model, the age by flow rate interaction term was observed to be a significant covariate for both stimulated (F=2.45, p<0.035) and unstimulated (F=2.42, p<0.035) submandibular/sublingual saliva. Also, when the THM-crown caries measure was predicted by the model, the age by flow rate interaction term was observed to be a significant covariate for both stimulated (F=2.45, p<0.035) and unstimulated (F=2.42, p<0.035) submandibular/sublingual saliva. Also, when the THM-crown caries measure was predicted by the model, the age by flow rate interaction term was observed to be a significant

covariate for stimulated (F=2.50, p<0.030) submandibular/sublingual saliva. For unstimulated parotid saliva, the only significant covariate for any caries measure was flow rate with tooth caries (F=4.68, p<0.035). None of the results for stimulated parotid saliva were significant. The limitation of this model is that it does not take the specific salivary components into consideration.

Flow rate concentration Model (Table 4): The concentration measures for specific salivary components are expressed as ug/ml or mEq/L. This model examines the significance of concentration as a covariate after controlling for age and flow rate and excludes the combined relation between flow rate and concentration, which are assumed to be statistically independent. For each combination of specific salivary component concentrations and caries measures, if the age-adjusted correlation between flow rate and concentration is significant than the age-adjusted correlation between that concentration and the caries measure, then model 2 would not be preferred since the assumption of statistical independence between flow rate and concentration is violated.

When the THM crown caries measure was predicted by the model, calcium concentration was observed to be a significant covariate after adjusting for age and flow rate for both unstimulated (F=7.42, p<0.010) and stimulated (F=4.32, p<0.040) submandibular/sublingual saliva. Similarly, after controlling for age and flow rate, calcium concentration of unstimulated submandibular/sublingual saliva was observed to be a significant covariate for predicting T (Tooth)-restorations (F=9.55, p<0.005). In each case, calcium concentration was shown to be uncorrelated with age-adjusted flow rate, supporting the Flow Rate – Concentration Model, and the age group by concentration interaction term was not significant (p>0.10). No other salivary

component concentrations were shown to be associated with caries measures while also being uncorrelated with flow rate.

Output Model (Table 5): The Output Model, which includes as covariates age group, salivary component output (the product of flow rate and concentration), and the interaction of age group with output, is an easier model to interpret than the Flow Rate – Concentration Model. Expressing salivary component data in terms of output also has the advantage of not requiring further validation of the model by investigating the association between flow rate and concentration. For unstimulated parotid saliva, the only model with significant results was for potassium output with root caries, with potassium output (F=5.00, p<0.030) and the interaction of age group with potassium output (F=5.48, p<0.001) shown to be significant covariates for root caries. No significant results were observed for stimulated parotid saliva.

The output measures of several components of unstimulated submandibular/sublingual saliva were observed to be significant covariates for DMFT and THM-crown and root caries measures, reflecting the strong associations observed for the Flow Rate Model. Protein output was a significant covariate for THM-crown (F=8.97, p<0.005), DMFT-crown (F=13.71, p<0.001), and DMFT-root (F=11.63, p<0.001). The interaction of protein output with age group was also a significant covariate for DMFT-crown (F=2.47, p<0.035) and DMFT-root (F=2.39, p<0.040). Other components having output measures that were significant covariates for THM-crown, THM-root, DMFT-crown, and DMFT-root included sodium (p<0.035) and calcium (p<0.005). Potassium output was a significant covariate for both DMFT-crown and DMFT-root (p<0.005). Both chloride output (F=11.28, p<0.001) and the interaction of age group with chloride output (F=2.45, p<0.035) were significant covariates for DMFT-root. Owing to the larger sample size, the models for stimulated submandibular/sublingual saliva had component output measures that

were almost always significant covariates for crown and root caries measures, which again reflected the results for the Flow Rate Model. Of the 12 saliva components examined (protein, sodium, potassium, chloride, calcium, lactoferrin, IgA, lysozyme, albumin, mucin1, mucin2, and cystatin), all but mucin2 had output measures that were significant covariates for THM-crown (p<0.005), while the interaction terms for age group with potassium (F=2.95, p<0.015), chloride (F=2.57, p<0.025), and calcium (F=3.07, p<0.010) were also significant covariates for THMcrown. All saliva components except lactoferrin and albumin had output measures that were significant covariates for THM-root (p < 0.030), and the age group by potassium (F=2.40, p<0.035) and chloride (F=3.00, p<0.015) output interaction terms were also significant covariates for THM-root. All 12 saliva components had output measures that were significant covariates for DMFT-crown (p<0.050) and DMFT-root (p<0.045). The interactions of age group with sodium (F=2.58, p<0.025), chloride (F=2.85, p<0.015), and albumin (F=3.25, p<0.010) output measures were also significant covariates for DMFT-crown, while the interactions of age group with protein (F=2.52, p<0.030), sodium (F=3.47, p<0.005), chloride (F=3.82, p<0.005), and albumin (F=4.60, p<0.001) output measures were also significant covariates for DMFT-root. In addition, there was one Output Model that did not involve crown or root caries measures for stimulated submandibular/sublingual saliva in which albumin output was observed to be a significant (F=14.79, p<0.001) covariate for tooth caries.

I. DISCUSSION

The purpose of this study was to explore, from the OH-SALSA data base, the relationship between salivary anticariogenic proteins controlled for flow rate, and dental caries in stratified age groups.

In this cross-sectional study there were no age-related effects on the flow rates of unstimulated

and stimulated parotid saliva. This finding has been supported by numerous other studies (35). In unstimulated parotid saliva, the only significant covariate for any caries measure was flow rate with coronal caries. The results of this study show that compared to parotid saliva, there are marked age-related changes in submandibular/sublingual saliva flow rate, composition and caries. Since submandibular/sublingual saliva comprises the majority of saliva in the mouth (36), these changes may have clinical implications.

In general, the results of this study show that the output measures of several components (total protein, sodium, calcium, chloride and potassium) of unstimulated submandibular/sublingual saliva were observed to be significant covariates for caries. The interactions of age group with certain components (sodium, chloride) were also significant covariates for the caries measures. The models for stimulated submandibular/sublingual saliva had component output measures (total protein, sodium, potassium, chloride, calcium, lactoferrin, IgA, lysozyme, albumin, mucin1, mucin2, and cystatin) that were almost always significant covariates for crown and root caries measures. The interactions of age group with certain components (potassium, chloride, calcium, lysozyme, mucin1, sodium and albumin) were also significant covariates for caries. The only other two studies reporting age related effects on submandibular/sublingual saliva composition, did not take caries into consideration (23, 24, and 25). This study adds the caries relationship to the understanding of age related function, and this appears to be the first such report for submandibular/sublingual saliva.

In a more specific age comparison of younger age groups (35-44years), and the oldest age group (75+ years) there is a direct (positive) relationship between total protein outputs, in stimulated and unstimulated submandibular/sublingual saliva, and both crown and root caries, with age (Table 6, 7, 12). There is a direct (positive) relationship between chloride outputs, in stimulated

and unstimulated submandibular/sublingual saliva, root caries, crown caries and the THM crown caries measure, with age (Table 8, 10, 14). There also exists a direct (positive) relationship between sodium outputs and albumin output, in stimulated submandibular/sublingual saliva, and both crown and root caries with age (Table 9, 11, 13, 15). These three sets of findings are in general agreement with most previous studies which indicate that there are age associated alterations in certain aspects of salivary gland function. Specifically in this study unstimulated and stimulated submandibular/sublingual saliva all decreased in these attributes with increasing age, and this is consistent with these prior reports. (23, 26, 29). Nevertheless studies of the Baltimore Longitudinal Study of Aging cohort generally report no change in flow rate for either stimulated or unstimulated submandibular/sublingual saliva (37). Therefore our interpretation of this study remains cautious.

J. CONCLUSION

In the function of these glands, significant associations were found between caries, age and specific individual proteins (Lactoferin, Albumin, Lysozyme, Mucin and Cystatin) and specific inorganic constituents (Potassium, Calcium, Sodium, and Chloride). The biologic understanding of these associations will be the subject of additional work.

K. CHANGES, WERE THE PROJECT TO BE REPEATED

1) Learning from this study, if repeated the salivary output model could be utilized more exclusively, greatly reducing the intensity and complexity of the multivariate analysis.

2) To limit analysis and to ease interpretation, the DMFT components appear to serve as suitable, stand alone caries measures, and could be exclusively used. The THM Index did not add to interpretation.

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M. APPENDIX

Table 1

Functions of saliva	in relation to salivary components involved
Functions	Salivary components involved
Lubrication	Mucins, Proline-rich proteins, water
Antimicrobial	Lactoferrin, Lysozyme, Lactoperoxidase, sIgA, Mucins, Histatins,
	Cystatins, Proline-rich proteins
Remineralization	Ca ²⁺ , PO ⁴⁻ , Pl, Statherins, Anionic proline-rich proteins
Cleansing	Water
Buffering	HCO3 ⁻ , PO4 ⁻
Digestive	Amylase, Lipase, Proteases, Water, Nucleases, Mucins, Gustin
Mucosal Integrity	Water, Electrolyte, Mucins

Table 2

Sur mitorio City, read Statistics and Den	Number	Danacat
	Number	Percent
San Antonio Population:	1144646	100.00%
Sex and Age		
Male	553245	48.33%
Female	591401	51.67%
Under 5 years	92446	8.08%
5 to 9 years	91849	8.02%
10 to 14 years	89113	7.79%
15 to 19 years	88951	7.77%
20 to 24 years	87684	7.66%
25 to 34 years	177842	15.54%
35 to 44 years	174810	15.27%
45 to 54 years	138880	12.13%
55 to 59 years	46898	4.1%
60 to 64 years	36811	3.22%
65 to 74 years	64108	5.6%
75 to 84 years	41707	3.64%
85 years and over	13547	1.18%
Median age (years)	31.7	
18 years and over	817989	71.46%
Male	386722	33.79%
Female	431267	37.68%
21 years and over	764332	66.77%
62 years and over	140740	12.3%
65 years and over	119362	10.43%
Male	47756	4.17%
Female	71606	6.26%

Race		
One race	1102775	96.34%
White	774708	67.68%
Black or African American	78120	6.82%
American Indian and Alaska Native	9584	0.84%
Asian	17934	1.57%
Asian Indian	3378	0.3%
Chinese	3271	0.29%
Filipino	3815	0.33%
Japanese	1267	0.11%
Korean	2102	0.18%
Vietnamese	2168	0.19%
Other Asian	1933	0.17%
Native Hawaiian and Other Pacific Islander	1067	0.09%
Native Hawaiian	306	0.03%
Guamanian or Chamorro	363	0.03%
Samoan	133	0.01%
Other Pacific Islander	265	0.02%
Some other race	221362	19.34%
Two or more races	41871	3.0 %
Hispanic or Latino and race		
Total Population	1144646	100.00%
Hispanic or Latino(of any race)	671394	58.66%
Mexican	473420	41.36%
Puerto Rican	7774	0.68%
Cuban	1491	0.13%
Other Hispanic or Latino	188709	16.49%
Not Hispanic or Latino	473252	41.34%
White alone	364357	31.83%

Parotid/			Flow Rate			Agegrp by Flow	Agegrp by Flow
Submand/		Caries	Partial	Flow Rate	Flow Rate	Interaction F-	Interaction p-
Sublingual	U/S	Measure	Corr	F-value	p-value	value	value
Р	U	T_CAR	-0.1138	4.677	0.031	0.701	0.623
S/S	U	THM_C	0.1562	16.47	0	0.46	0.806
S/S	U	THM_R	0.1102	11.345	0.001	0.618	0.686
S/S	U	DMF_C	-0.1519	19.298	0	1.706	0.13
S/S	U	DMF_R	-0.1549	22.778	0	2.417	0.034
S/S	S	THM_C	0.01599	13.411	0	2.495	0.03
S/S	S	THM_R	0.1337	10.935	0.001	2.089	0.064
S/S	S	DMF_C	-0.1502	15.319	0	1.825	0.105
S/S	S	DMF_R	-0.1546	17.401	0	2.45	0.032

Table 3: Flow Rate Model

P:	Parotid Gland
S/S:	Submandibular/Sublingual gland
U/S:	Unstimulated and Stimulated saliva
T_CAR:	Tooth Caries i.e. DT of Crown
R_CAR:	Root Caries i.e. DT of Root
THM_C:	Tissue Health Index for Crown Caries
THM_R:	Tissue Heath Index for Root Caries
DMF_C:	Crown Caries
DMF_R:	Root Caries

Table 4: Flow Rate Concentration Model

				Conc			Agegrp by Conc	Agegrp by Conc
Parotid/		Caries	Conc	Partial	Conc	Conc	Interaction	Interaction
Submand	U/S	Measure	Measure	Corr	F-value	p-value	F-value	p-value
Р	S	T_RES	Chloride	-0.0769	6.719	0.01	0.642	0.667
Р	S	DMF_R	Potassium	0.0738	4.597	0.032	0.834	0.525
S/S	U	THM_C	Calcium	0.0876	7.423	0.007	1.643	0.146
S/S	U	T_RES	Calcium	-0.0977	9.552	0.002	1.102	0.358
S/S	S	THM_C	Calcium	0.0782	4.318	0.038	0.068	0.997
S/S	S	THM_R	Mucin1	0.0713	10.19	0.001	1.798	0.111
S/S	S	T-RES	Mucin1	0.0697	4.032	0.045	0.69	0.631
S/S	S	T_CAR	Albumin	0.1727	15.843	0	1.57	0.166
S/S	S	THM_C	Cystatin	0.0838	4.331	0.038	0.066	0.977

P:	Parotid Gland
S/S:	Submandibular/Sublingual gland

U/S:	Unstimulated and Stimulated saliva
T_CAR:	Tooth Caries i.e. DT of Crown
R_CAR:	Root Caries i.e. DT of Root
THM_C:	Tissue Health Index for Crown Caries
THM_R:	Tissue Heath Index for Root Caries
DMF_C:	Crown Caries
DMF_R:	Root Caries
T_RES:	Tooth Restoration

Table 5: Output Model

Parotid/		Caries	Output	Output	Output	Output	Agegrp by Output	Agegrp by Output	Age group
Submand	U/S	e	Measure	Corr	F-value	value	F-value	value	
Р	U	R_CAR	Potassium	0.2882	5.003	0.029	5.48	0.001	.001
S/S	U	THM_C	Protein	0.0997	8.965	0.003	1.17	0.322	.000
S/S	U	THM_C	Sodium	0.1208	7.853	0.005	1.241	0.288	.000
S/S	U	THM_C	Calcium	0.1446	11.659	0.001	0.868	0.502	.000
S/S	U	THM_R	Sodium	0.1063	10.684	0.001	0.941	0.453	.000
S/S	U	THM_R	Calcium	0.1008	9.805	0.002	0.445	0.817	.000
S/S	U	DMF_C	Protein	-0.1165	13.712	0	2.47	0.031	.000
S/S	U	DMF_C	Sodium	-0.0742	4.545	0.033	1.16	0.327	.000
S/S	U	DMF_C	Potassium	-0.1024	9.286	0.002	0.834	0.526	.000
S/S	U	DMF_C	Calcium	-0.1383	15.149	0	0.783	0.562	.000
S/S	U	DMF_R	Protein	-0.1099	11.629	0.001	2.389	0.036	.000
S/S	U	DMF_R	Sodium	-0.0937	8.727	0.003	1.719	0.128	.000
S/S	U	DMF_R	Potassium	-0.1078	9.304	0.002	1.074	0.373	.000
S/S	U	DMF_R	Chloride	-0.0927	11.284	0.001	2.452	0.032	.000
S/S	U	DMF_R	Calcium	-0.1414	15.256	0	1.112	0.353	.000
S/S	S	THM_C	Protein	0.1654	17.452	0	2.156	0.057	.000
S/S	S	THM_C	Sodium	0.1354	9.979	0.002	1.52	0.181	.000
S/S	S	THM_C	Potassium	0.1512	13.004	0	2.946	0.012	.000
S/S	S	THM_C	Chloride	0.1213	6.524	0.001	2.571	0.025	.000
S/S	S	THM_C	Calcium	0.199	25.062	0	3.069	0.009	.000
S/S	S ·	THM_C	Lactoferrin	0.0928	7.775	0.005	1.289	0.266	.000
S/S	S	THM_C	IgA	0.1214	11.482	0.001	0.127	0.986	.000
S/S	S	THM_C	Lysozyme	0.1009	17.636	0	2.228	0.05	.000
S/S	S	THM_C	Albumin	0.1389	17.636	0	2.228	0.05	.000
S/S	S	THM_C	Mucin1	0.1172	9.028	0.003	2.453	0.032	.000
S/S	S	THM_C	Cystatin	0.1686	19.944	0	1.111	0.352	.000
S/S	S	THM_R	Protein	0.1314	12.797	0	0.1225	0.295	.000
S/S	S	THM_R	Sodium	0.128	9.869	0.002	2.037	0.071	.000
S/S	S	THM_R	Potassium	0.1417	14.382	0	2.402	0.035	.000
S/S	S	THM_R	Chloride	0.1092	6.425	0.011	2.995	0.011	.000
S/S	S	THM_R	Calcium	0.1502	15.654	0	2.013	0.074	.000
S/S	S	THM_R	IgA	0.0943	6.934	0.009	0.295	0.916	.000
S/S	S	THM_R	Lysozyme	0.0751	6.26	0.013	1.687	0.135	.000
S/S	S	THM_R	Mucin1	0.1375	16.132	0	1.188	0.313	.000

S/S	S	THM_R	Mucin2	0.077	4.767	0.029	1.096	0.361	.000
S/S	S	THM_R	Cystatin	0.1255	13.809	0	0.496	0.779	.000
S/S	S	T_CAR	Albumin	0.1369	14.785	0	1.538	0.175	.000
S/S	S	DMF_C	Protein	-0.1357	13.66	0	1.658	0.142	.000
S/S	S	DMF_C	Sodium	-0.1018	5.903	0.015	2.584	0.025	.000
S/S	S	DMF_C	Potassium	-0.1324	14.71	0	1.052	0.386	.000
S/S	S	DMF_C	Chloride	-0.093	3.954	0.047	2.848	0.015	.000
S/S	S	DMF_C	Calcium	-0.17	22.62	0	0.998	0.418	.000
S/S	S	DMF_C	Lactoferrin	-0.0826	8.587	0.003	0.931	0.46	.000
S/S	S	DMF_C	IgA	-0.1145	15.048	0	0.649	0.662	.000
S/S	S	DMF_C	Lysozyme	-0.0725	6.404	0.012	0.915	0.471	.000
S/S	S	DMF_C	Albumin	-0.1407	25.65	0	3.246	0.006	.000
S/S	S	DMF_C	Mucin1	-0.084	7.479	0.006	1.786	0.113	.000
S/S	S	DMF_C	Mucin2	-0.0875	4.78	0.029	0.646	0.665	.000
S/S	S	DMF_C	Cystatin	-0.1318	15.979	0	0.986	0.425	.000
S/S	S	DMF_R	Protein	0.1367	13.564	0	2.524	0.028	.000
S/S	S	DMF_R	Sodium	-0.1084	7.171	0.008	3.466	0.004	.000
S/S	S	DMF_R	Potassium	0.1371	15.733	0	1.547	0.172	.000
S/S	S	DMF_R	Chloride	-0.0922	4.192	0.041	3.815	0.002	.000
S/S	S	DMF_R	Calcium	-0.162	21.438	0	1.623	0.151	.000
S/S	S	DMF_R	Lactoferrin	-0.0818	7.823	0.005	1.032	0.397	.000
S/S	S	DMF_R	IgA	-0.1245	14.166	0	0.592	0.707	.000
S/S	S	DMF_R	Lysozyme	0.0686	5.154	0.023	1.499	0.187	.000
S/S	S	DMF_R	Albumin	-0.1234	25.567	0	4.598	0	.000
S/S	S	DMF_R	Mucin1	-0.1014	10.251	0.001	1.125	0.346	.000
S/S	S	DMF_R	Mucin2	-0.0822	5.196	0.023	0.627	0.679	.000
S/S	S	DMF_R	Cystatin	-0.1336	17.005	0	2.168	0.055	.000

P:	Parotid Gland
S/S:	Submandibular/sublingual gland
U/S:	Unstimulated and Stimulated saliva
T_CAR:	Tooth Caries i.e. DT of Crown
R CAR:	Root Caries i.e. DT of Root
THM_C:	Tissue Health Index for Crown Caries
THM R:	Tissue Heath Index for Root Caries
DMF_C:	Crown Caries
DMF_R:	Root Caries
T RES:	Tooth Restoration

			95% Confider	nce Interval
AGEGRP	Mean	Std. Error	Lower Bound	Upper Bound
35 - 44	9.681(a)	.634	8.437	10.926
45 - 54	10.624(a)	.460	9.722	11.525
55 - 64	12.135(a)	.442	11.268	13.001
65 - 69	13.934(a)	.470	13.012	14.856
70 - 74	14.283(a)	.478	13.344	15.222
75+	15.780(a)	.880	14.054	17.506

Table 6: Protein (Unstimulated Submandibular/Sublingual):

a Covariates appearing in the model are evaluated at the following values: output pross = .7993.

Table 7: Protein (Unstimulated Submandibular/Sublingual):

Dependent Variable: dmf-r

		Std. Error	95% Confidence Interval	
AGEGRP	Mean		Lower Bound	Upper Bound
35 - 44	2.166(a)	.719	.756	3.577
45 - 54	3.853(a)	.533	2.807	4.898
55 - 64	6.373(a)	.560	5.274	7.471
65 - 69	9.659(a)	.598	8.484	10.834
70 - 74	10.360(a)	.644	9.095	11.625
75+	11.967(a)	1.013	9.980	13.955

a Covariates appearing in the model are evaluated at the following values: output prous = .4930.

Table 8: Chloride (Unstimulated Submandibular/Sublingual): Dependent Variable: dmf-r

AGEGRP		Std. Error	95% Confidence Interval	
	Mean		Lower Bound	Upper Bound
35 - 44	2.078(a)	.718	.669	3.487
45 - 54	3.854(a)	.534	2.806	4.901
55 - 64	6.369(a)	.560	5.269	7.468
65 - 69	9.692(a)	.600	8.516	10.869
70 - 74	10.607(a)	.639	9.352	11.861
75+	11.448(a)	1.066	9.357	13.539

a Covariates appearing in the model are evaluated at the following values: output clus = .0391.

Table 9: Sodium (Stimulated Submandibular/Sublingual): Dependent Variable: dmf-c

			95% Confidence Interval	
AGEGRP	Mean	Std. Error	Lower Bound	Upper Bound
35 - 44	9.698(a)	.675	8.373	11.023
45 - 54	10.713(a)	.465	9.801	11.625
55 - 64	12.165(a)	.444	11.293	13.036
65 - 69	13.863(a)	.487	12.908	14.818
70 - 74	14.214(a)	.490	13.253	15.175
75+	16.596(a)	.920	14.791	18.402

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a Covariates appearing in the model are evaluated at the following values: output nass = .0650.

Table 10: Chloride (Stimulated Submandibular/Sublingual): Dependent Variable: dmf-c

			95% Confidence Interval	
AGEGRP	Mean	Std. Error	Lower Bound	Upper Bound
35 - 44	9.626(a)	.655	8.340	10.912
45 - 54	10.684(a)	.460	9.781	11.586
55 - 64	12.098(a)	.442	11.230	12.965
65 - 69	13.812(a)	.480	12.869	14.754
70 - 74	14.295(a)	.481	13.351	15.238
75+	16.489(a)	.900	14.723	18.255

a Covariates appearing in the model are evaluated at the following values: output clss = .0688.

Table 11: Albumin (Stimulated Submandibular/Sublingual): Dependent Variable: dmf-c

	Mean	Std. Error	95% Confidence Interval	
AGEGRP			Lower Bound	Upper Bound
35 - 44	9.446(a)	.612	8.246	10.647
45 - 54	10.654(a)	.453	9.766	11.543
55 - 64	12.142(a)	.441	11.276	13.008
65 - 69	13.827(a)	.472	12.901	14.753
70 - 74	14.532(a)	.468	13.613	15.450
75+	14.927(a)	.849	13.261	16.593
a Covariates app	earing in the model	are evaluated at	the following values: or	utput albss = 1.0666

Table 12: Protein (Stimulated Submandibular/Sublingual): Dependent Variable: dmf-r

			95% Confidence Interval	
AGEGRP	Mean	Std. Error	Lower Bound	Upper Bound
35 - 44	2.325(a)	.727	.899	3.750
45 - 54	4.254(a)	.526	3.221	5.287
55 - 64	6.663(a)	.506	5.670	7.655
65 - 69	10.031(a)	.538	8.976	11.087
70 - 74	10.851(a)	.548	9.776	11.926
75+	12.012(a)	1.007	10.035	13.989

a Covariates appearing in the model are evaluated at the following values: output pross = .7993.

Table 13: Sodium (Stimulated Submandibular/Sublingual): Dependent Variable: dmf-r

			95% Confidence Interval	
AGEGRP	Mean	Std. Error	Lower Bound	Upper Bound
35 - 44	2.180(a)	.777	.656	3.704
45 - 54	4.390(a)	.535	3.341	5.439
55 - 64	6.723(a)	.511	5.720	7.726
65 - 69	9.848(a)	.560	8.749	10.947
70 - 74	10.748(a)	.563	9.643	11.854
75+	12.749(a)	1.058	10.673	14.826

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a Covariates appearing in the model are evaluated at the following values: output nass = .0650.

Table 14: Chloride (Stimulated Submandibular/Sublingual): Dependent Variable: dmf-r

			95% Confidence Interval	
AGEGRP	Mean	Std. Error	Lower Bound	Upper Bound
35 - 44	2.148(a)	.751	.675	3.622
45 - 54	4.303(a)	.527	3.269	5.338
55 - 64	6.621(a)	.506	5.628	7.615
65 - 69	9.852(a)	.550	8.773	10.932
70 - 74	10.860(a)	.551	9.779	11.941
75+	12.788(a)	1.031	10.765	14.811

a Covariates appearing in the model are evaluated at the following values: output clss = .0688.

 Table 15:
 Albumin (Stimulated Submandibular/Sublingual):

 Dependent Variable: dmf-r

			95% Confidence Interval	
AGEGRP	Mean	Std. Error	Lower Bound	Upper Bound
35 - 44	2.114(a)	.701	.739	3.488
45 - 54	4.364(a)	.518	3.347	5.381
55 - 64	6.732(a)	.505	5.740	7.723
65 - 69	9.884(a)	.540	8.823	10.944
70 - 74	11.172(a)	.536	10.121	12.223
75+	10.475(a)	.972	8.568	12.382

a Covariates appearing in the model are evaluated at the following values: output albss = 1.0666.