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# Total Salivary Protein Concentration and its Correlation to Dental Caries

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# Total Salivary Protein Concentration and its Correlation to Dental Caries

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**ABSTRACT:** According to the World Health Organization, dental cavities are the number one chronic disease in children. Saliva coats the teeth all day and serves many functions to maintain and protect teeth. Saliva has many proteins that can be both detrimental and essential to the preservation of tooth enamel. The purpose of this study was to determine if a correlation exists between the total protein concentration in saliva and the prevalence of cavities in the mouth. We hypothesized that there would be a positive correlation between total salivary protein concentration and the prevalence of cavities in the participant. Saliva samples were collected from patients during their comprehensive exams at the University of Central Florida (UCF) Dental Center and were analyzed using the DC assay to determine the protein concentration. These results were compared to the number of cavities found in each patient's mouth to determine if a correlation exists between protein concentration and cavity number. The correlation between the variables was weak, indicating that salivary protein concentration and cavity number are not significantly related. These results suggest that total salivary protein concentration alone may not be a sufficient diagnostic marker in determining the likelihood of cavities. This may be due to the multifactorial nature of cavity formation, but further research is needed to confirm this.

**KEYWORDS:** dental; dentistry; teeth; cavities; caries; protein; saliva; mouth; hygiene; health; medicine

**THE PEGASUS REVIEW:**
UNIVERSITY OF CENTRAL FLORIDA
UNDERGRADUATE RESEARCH JOURNAL

URJ

**12.2:** 26-33

## INTRODUCTION

According to the World Health Organization, dental cavities are the number one chronic disease in children. Worldwide, nearly 60-90% of school children have dental cavities, which can lead to pain, discomfort, infection, and even systemic issues (World Health Organization, 2012).

Oral diseases do not stop at the mouth. The oral cavity is the main entryway to the rest of the body, and microbes can travel through the oral cavity and affect systemic health (Gray & Lewis, 2000; Li, Kolltveit, Tronstad, & Olsen, 2000). Oral infection has been linked to endocarditis, myocarditis, and orbital cellulitis (Li, Kolltveit, Tronstad, & Olsen, 2000). In 2007, an uninsured twelve-year-old boy died because the bacteria from a dental abscess spread to his brain (Gallagher, 2018).

Tooth decay is mostly preventable through fluoride treatments, professional cleanings, sealants, and following proper hygiene techniques at home. Keeping up with oral health routines by visiting a dentist twice a year is essential to preventing oral diseases. The American Dental Association recommends brushing twice a day, flossing once a day, and seeing the dentist regularly in order to prevent dental disease (American Dental Association, 2001). More frequent visits to the doctor allows for earlier preventative measures and earlier diagnoses, which can keep the cost of their treatment low and help to maintain a patient's health.

The mouth encounters many different substances and microbes. Accordingly, the oral cavity has many different defense mechanisms including the anatomy, oral microbiome, immune system, and saliva. Enamel, made of hydroxyapatite, is the hardest substance in the human body (Harris, Garcia-Godoy, & Nathe, 2014). Enamel is constantly being remineralized and demineralized, meaning that calcium, phosphate, and other ions are being removed and added. Acidity leads to the demineralization of the enamel, as seen in Figure 1, and enamel kept in an acidic environment for too long without enough time to remineralize can lead to caries formation.

Plaque is a substance that adheres onto the surfaces of teeth and provides a scaffold for bacteria. Plaque's potential to harm the enamel of the tooth depends on the microbes living inside of it (Kolenbrander, et al., 2000). Plaque formation starts with an acquired pellicle, which is made from mostly glycoproteins from the saliva as well as carbohydrates (Harris, Garcia-Godoy, & Nathe, 2014). Bacteria then begin to colonize the acquired pellicle, forming a biofilm, which then becomes plaque. Plaque can house over 150 different species of microbes that come from the environment randomly (Harris, Garcia-Godoy, & Nathe, 2014). Microorganisms living in the biofilm can produce a slime layer to protect themselves from being dislodged from the tooth as well as fibrils or

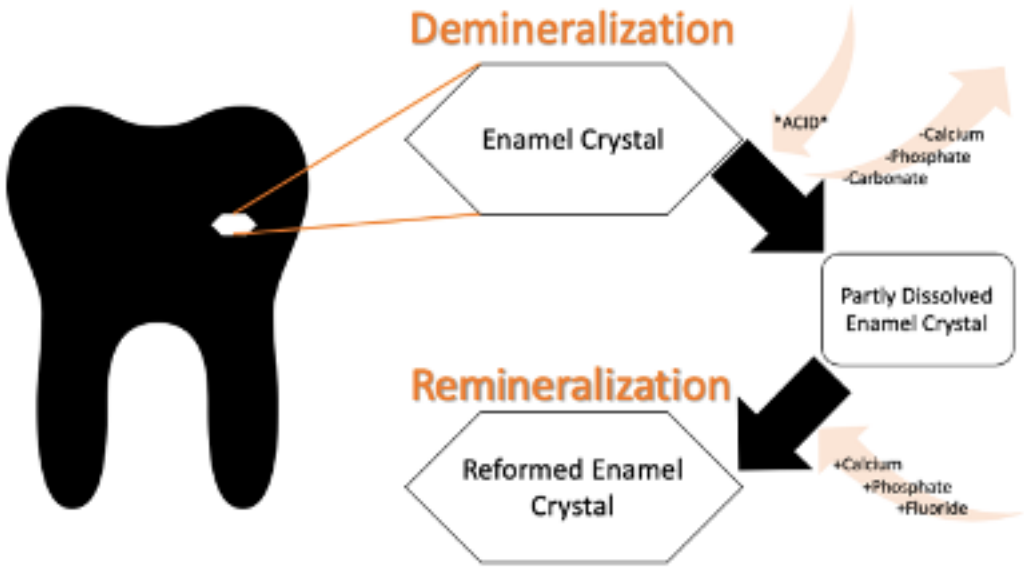


*Figure 1. Enamel Demineralization and Remineralization*

THE PEGASUS REVIEW:
UNIVERSITY OF CENTRAL FLORIDA
UNDERGRADUATE RESEARCH JOURNAL

URJ

12.2: 26-33

appendages which aid in attachment to the enamel. It only takes two hours to make a plaque, two days for it to double in size, and 21 days for it to become so large that the microorganisms inside can no longer have access to oxygen, resulting in metabolism switching to anaerobic glycolysis (Baier & Glantz, 289-30; Tanzer & Johnson, 1976; Marsh, 1999). Anaerobic glycolysis occurs because oxygen cannot cross more than 0.1 mm into the plaque (Van der Hoeven, de Jong, & Kolenbrander, 1985; Globerman & Kleinberg, 1979). Bacterial anaerobic glycolysis has been shown to produce acidic byproducts capable of dropping the pH in its environment from 7.5 to 4.6, which can be detrimental since enamel demineralizes at a pH of 5.5 (Harris, Garcia-Godoy, & Nathe, 2014).

Saliva is a mucosal secretion released in the mouth by salivary glands. Saliva has multiple functions, including lubrication, flushing of microbes and plaque, holding chemicals, aiding with antimicrobial processes, maintaining a calcium and phosphorus concentration to help with remineralization of enamel, and buffering of acidogenesis (Dowd, 1995; Lageroff, 1998). There are three major salivary glands: the parotid gland which secretes by the upper second molars, the sublingual gland, which secretes under the tongue, and the submandibular gland, which also secretes under the tongue. There are also many minor salivary glands found throughout the mouth that aid in mastication (Harris, Garcia-Godoy, & Nathe, 2014). These glands can produce either serous or mucous secretions, and the concentrations of proteins they secrete can vary. However, studying whole saliva is the most relevant technique because it coats the teeth (Rudney, Krig, Neuvar, Soberay, & Iverson, 1991).

Salivary proteins can have different purposes. The four main salivary protein interactions studied are aggregation, adherence, cell killing, and nutrition (Scannapieco, 1994). Many studies of these four protein interactions are limited in that most are performed *in vitro*, and it is difficult to confirm these interactions *in vivo*. Aggregation *in vitro* has been shown to help form pellicles that are thought to possibly clear bacteria out of suspension, but this hypothesis has not been confirmed *in vivo* (Scannapieco, 1994). Adherence could help the bacteria bind to the pellicle and allow for colonization. The cell killing property of proteins can assist with fighting microbes in the pellicle and inhibit their metabolic activity. Nutritionally, salivary proteins can also help break down complex macromolecules for usage by bacteria when the host is in a fasting state.

Salivary proteins have been well studied and characterized, but their role in the formation of dental carries has not been definitively identified. Secretory IgA is a well-studied salivary protein that does not activate complement, but helps promote aggregation (Liljemark, Bloomquist, & Ofstehage, 1979). Lysozyme is a muramidase that assists with killing bacteria by lysing bacterial cell walls. It has also been shown to aid in aggregation and adherence (Golub, Cheruka, Boosz, Davis, & Malamud, 1985; Tellefson & Germaine, 1986). Lactoferrin is a protein that can sequester iron, which may be used as a means of inducing bacteriostasis or as a source of iron by bacteria (Arnold, Russell, Champion, Brewer, & Gauthier, 1982; Herrington & Sparling, 1985). This function depends on whether the iron is stored to sequester or supply the bacteria with iron for metabolism. Lactoferrin can also aid in aggregation and adherence (Soukka, Tenovuo, & Rundegren, 1993). Glycoproteins can assist with aggregation in solution or adherence to pellicles (Rudney J. D., 1995). Acidic proline-rich proteins can undergo a conformational change when absorbed by hydroxyapatite and expose other epitopes that can lead to adherence of oral bacteria to the pellicle and allow for colonization (Gibbons, Hay, & Schlesinger, 1991). Amylase can bind to oral *Streptococci* and aid in adherence to the pellicle as well as digestion of starch from the host, which microbes use as a source of energy (Scannapieco, Torres, & Levine, 1993). Due to limited time and resources for the study, the researchers took a broader approach, and total protein concentrations rather than specific proteins were analyzed.

In a study completed by Vibhakar, Patankar, Yadav, & Vibbhakar, thirty-nine patients had saliva samples collected, and the samples were analyzed for protein concentration. The total salivary protein levels showed a positive correlation with the Decayed, Missing, Filled Total teeth (DMFT) index (Vibhakar, Patankar, Yadav, & Vibbhakar, 2013). The DMFT index encodes how many teeth have had and/or presently have cavities. A poor, but positive relationship was found between the total salivary protein concentration and the number of teeth that had dental caries. For future studies, the authors suggested a larger sample size to confirm the data and further analysis into specific salivary proteins and the roles they play, whether it be protective or detrimental.

In dentistry, there are no set diagnostic measures for predicting the prevalence of cavities an individual may have. This study aims to explore the relationship between total salivary protein and the prevalence of cavities.

THE PEGASUS REVIEW:
UNIVERSITY OF CENTRAL FLORIDA
UNDERGRADUATE RESEARCH JOURNAL

URJ

12.2: 26-33

Investigation of this relationship will aid in the goal of one day having a salivary diagnostic test for examining the risk of an individual developing cavities and using this information to provide more preventative care for patients.

## METHODS

The Decayed, Missing, Filled Surface (DMFS) index encodes how many surfaces of the participants' teeth have had or presently have decay, are missing, or are filled due to decay. If there is a filling present that was done for a cosmetic purpose, it is not counted towards the DMFS index (Lo, 2019). When excluding wisdom teeth, which we did for our study, the maximum value an individual could have for DMFS is 140. A higher score on the DMFS index means that the participant has had more cavities that have been addressed and/or presently has cavities that need to be addressed. In this study, the DMFS index was used to correlate the protein concentration to the prevalence of caries in the participant.

The DC Protein Assay uses alkaline copper tartrate solution to bind copper to the peptide bonds of a protein's polypeptide chain. Folin reagent is then added, which binds to the peptide-copper complex to form a blue product with a maximum absorbance at 750 nm. We measured the absorbance of our samples using this method by comparing the values we receive to a standard curve.

Participants consisted of 43 UCF students and faculty between the ages of 18-29 who had presented to Student Health Services for a dental appointment and had a comprehensive or periodic exam completed in 2018 or 2019. Patients who were pregnant, frequent smokers, or had oral pathologies such as cancer or periodontitis, which could affect their salivary protein concentrations, were excluded from the study. During their dental visit, participants were asked to rinse their mouth with water to remove food residue and then waited 10 minutes after rinsing to avoid sample dilution before the collection of saliva via drooling. Sterile containers were used to obtain a 0.5-1 mL sample of saliva and the DMFSI number was diagnosed by the doctor. At the end of the collection period, samples were stored in a freezer maintained at -20°C until the DC Protein Assay was performed.

Samples were thawed out on ice, resuspended by vortexing, and then centrifuged at 17,000 x g for 25 minutes to pellet out any blood, bacteria, or food debris that may have been collected. The DC protein assay was then performed using a serial dilution of bovine serum albumin standard curve at concentrations 4, 8, 12, 16, and 20 mg/mL. The absorbance of the saliva samples was then measured and plugged into the standard curve equation to determine the protein concentration.

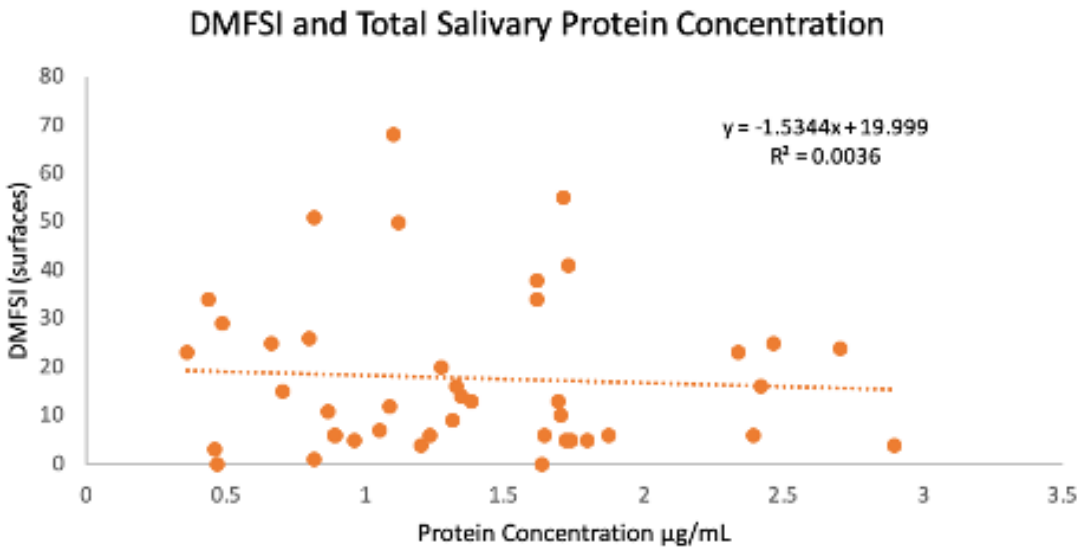


*Figure 2. DMFSI vs Total Protein with linear regression model.*

THE PEGASUS REVIEW:
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UNDERGRADUATE RESEARCH JOURNAL

URJ

12.2: 26-33

## RESULTS

The Decayed, Missing, Filled Surface (DMFS) index and total protein concentration were determined for each of the 43 samples. The result of linear regression analysis showed that the relationship between total protein concentration and DMFSI was not significant ($R^2$=0.0036, Figure 2). We split the patients into 3 groups according to their total protein concentration: those with protein concentration ranging 0-1 µg/mL (defined as low), >1-2 µg/mL (medium), and >2 µg/mL (high). We then performed an ANOVA test. The ANOVA result showed that the average DMSI was not significantly different among the 3 groups ($F$ = 0.11, $p$ = 0.90, Table 1, Figure 3).

The results of the ANOVA test showed that our p-value was 0.90. Additionally, the F statistic, which measures the ratio of between-group to within-group MS, was 0.11, indicating that the observed variance in DMFSI was primarily due to random error that we did not control. This analysis demonstrates that there is more variation in DMFSI within each group than between groups. Given our current data, we cannot determine any significant association between total salivary protein and DMFSI.

## DISCUSSION

This research served to determine if a correlation could be observed between total salivary protein concentration and the prevalence of cavities in an individual. Based on the sample size and experimental limitations, there was no significant trend in our data. The correlation between the variables under investigation was not as strong as that found by prior research completed by Vibhakar, Patankar, Yadav, and Vibbhakar (2013). After meeting with a biostatistician, we hypothesize that this result may have been due to the insufficient amount of information collected from participants and the small sample size of 43 participants.

Even though our study focused on the linear relationship of total salivary protein concentration and the prevalence of cavities, other factors outside of our interests should have been collected as potential confounding variables. Variables such as age, gender, and time of collection could have been used in a multiple linear regression model as variables that we could not control at the sampling stage. Our current data failed to show a significant linear relationship because: (1) we failed to control for confounding variables; (2) the $R^2$ value was too small to

**Anova: Single Factor**

**SUMMARY**

| Groups | # of Samples | Sum | Average | Variance | Standard Error |
|---|---|---|---|---|---|
| 0-1 µg/mL | 14 | 235 | 16.7857143 | 225.873626 | 4.016693971 |
| >1-2 µg/mL | 23 | 437 | 19 | 358.636364 | 3.948782771 |
| >2-3 µg/mL | 6 | 98 | 16.3333333 | 87.4666667 | 3.818085617 |

**ANOVA**

| Source of Variation | SS | df | MS | F | P-value | F crit |
|---|---|---|---|---|---|---|
| Between Groups | 59.937430 | 2 | 29.968715 | 0.106425 | 0.8992953 | 3.231727 |
| Within Groups | 11263.690 | 40 | 281.59226 | | | |
| Total | 11323.627 | 42 | | | | |

*Table 1. Results of Anova Test showing Data Analysis*

**THE PEGASUS REVIEW:**
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**12.2:** 26-33

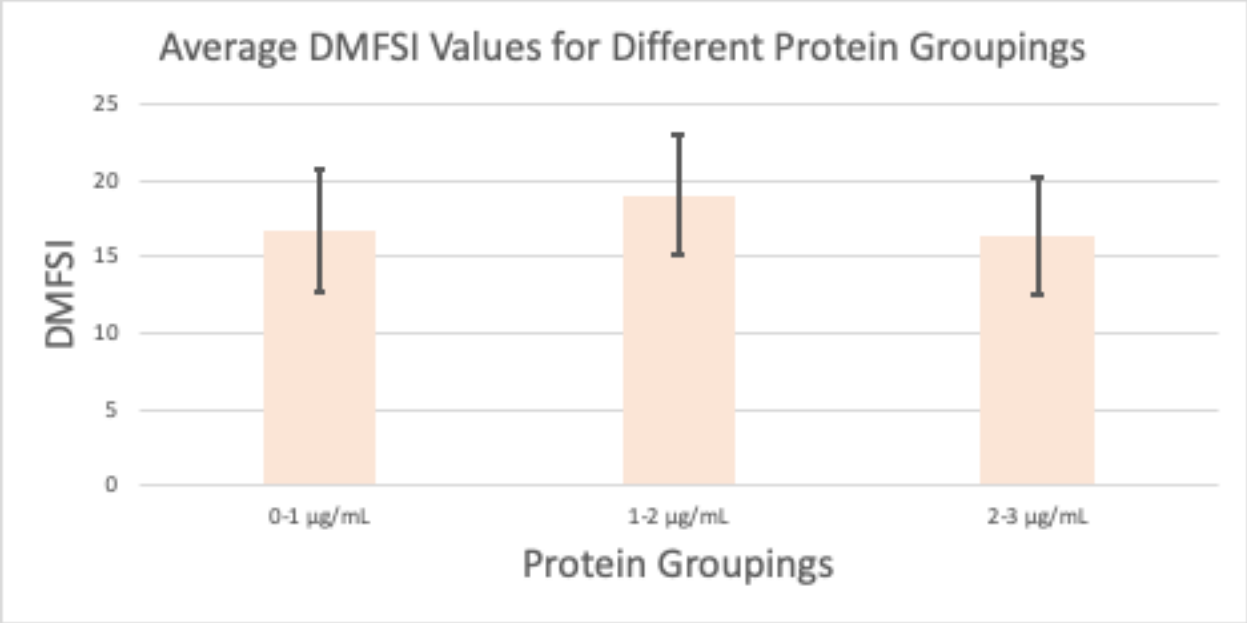


*Figure 3. Protein Concentration Groupings and DMFSI Values with Standard Error*

detect with such a small sample size; or (3) there is not a linear relationship between DMFS and total protein concentration.

To eventually develop a diagnostic salivary test for patient's caries risk, future studies would need to look at specific proteins in the saliva, as well as include more demographic information from participants. Future studies could increase the sample size and focus on more specific proteins and analyze the specific mechanisms of action that can lead to caries development.

## CONCLUSION

The results of this experiment revealed the need for more factors to be taken into account when comparing total protein to DMFSI. Future studies should include the age of participants as well as the time of day that samples are collected as this could affect the salivary protein concentration. Narrowing the scope of the study to investigate a specific protein and its relationship to DMFSI may also produce better results.

This was a general study to look at one possible factor that could contribute to dental caries formation. Once enough research has been completed and we have a better understanding of these mechanisms, diagnostic measures for those specific proteins as a marker for risk of caries prevalence can be developed. These tools could allow for improved preventative dentistry, reducing the need for restorative dentistry and allowing individuals to live healthier lives overall.

**THE PEGASUS REVIEW:**
UNIVERSITY OF CENTRAL FLORIDA
UNDERGRADUATE RESEARCH JOURNAL

URJ

**12.2:** 26-33

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**THE PEGASUS REVIEW:**
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UNDERGRADUATE RESEARCH JOURNAL

URJ

**12.2:** 26-33

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