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Original Article

Assessment of DMFT Score, Salivary Protein, and Flow Rate in UiTM's Year One Dental Students

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ABSTRACT

Objectives: *The aim of the study was to evaluate the year one dental students for their score of decayed, missing, filled teeth (DMFT), total salivary protein, and unstimulated and stimulated flow rate of salivary secretions. In addition, correlations between DMFT index with their salivary parameters were also elucidated in our study.* **Methods:** *Fifty-seven of dental students were selected in the study, and informed consents were obtained prior to study. Intraoral examination specifically the DMFT scoring was performed. Both salivary proteins in unstimulated and stimulated saliva were measured using Bicinchoninic acid protein assay, and finally flow rates of unstimulated and stimulated saliva were calculated in the study. Data obtained for DMFT score and salivary parameters were analyzed by descriptive test and Spearman's correlation test using SPSS version 26.0. Software Program (IBM, New York), Statistical significance was set at $p < 0.05$.* **Results:** *Majority subjects showed DMFT index of 40.6% in DMFT 1-3 and 36.9% in DMFT 4-10 respectively. Statistically significant inverse correlations were observed between salivary total protein and unstimulated salivary flow rate ($r=-0.314$, $p=0.017$), and DFMT score with stimulated salivary flow rate ($r=-0.244$, $p=0.067$).* **Conclusion:** *In our study, majority of first year dental students evidenced DMFT scores of 1-10, with slightly significant associated dental caries prevalence with salivary flow rate. The findings obtained may serve as reference values for the growing interest in saliva as a diagnostic tool in predicting dental caries risk.*

Keywords: *DMFT score; Total protein; Unstimulated saliva; Stimulated saliva*

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INTRODUCTION

World Health Organization (WHO) reported that 2.3 billion people suffer from dental caries of permanent teeth with more than 530 million children suffer from caries (2020). Dental caries remains as the major public health problem despite the preventive strategies mostly endorsed in developed countries (2020).

Saliva has an important role in preserving healthy hard and soft oral tissues. It contains essential components include proteins, electrolytes, and enzymes for host protection as stated by Pitts et al. (2017) and Helen et al. (2012). It also possesses defense mechanisms in protecting oral tissues by constant flow of saliva from mouth to the gut as asserted by Maddu & Gokul (2019). Besides that, it keeps the ecological equilibrium between the host and oral microbiota in a symbiotic state, Lynge & Belstrom (2019). In addition, Eva et al. (2019) and Stefan (2012) revealed that lysozyme, lactoferrins, immunoglobulins and albumins are salivary proteins that engage in the protection of oral tissues.

The range of protein concentration in normal healthy saliva is between 0.5-2.0 mg/mL claimed Mohamed et al, (2012). The most common major salivary glycoproteins that are expressed in saliva includes mucin, proline-rich protein (PRP) and immunoglobulins in which each hold the function of protecting teeth from enamel demineralization, remineralization and microbial binding respectively as stated Nireeksha et al. (2017) and Romilla et al. (2020) in their study. PRPs, the largest groups of salivary proteins, are sustaining tooth integrity by becoming part of the dental pellicle, George (2015), in a study done by Gao et al. (2016) also shown to be associated with caries-free subjects. Hence, the unbalance of salivary proteins level and its component will eventually disrupt the usual oral ecosystem. A previous study done by Hemadi et al. (2017), reported that the deficient association between dental caries and salivary total protein is due to the different levels of their structures and function redundancies in saliva. Other study showed a high level of total salivary protein in a caries free group compared to caries active group and salivary proteins concentrations may correlate with parallel increment of flow rate claimed Laputkova et al. (2014).

Individuals with low salivary flow may develop dry mouth problems with difficulty of eating, swallowing, speech, and poor oral hygiene stated in a review by Fatima et al. (2020). Two studies revealed that both stimulated and unstimulated salivary flow rates of submandibular or sublingual glands decreased with increasing age, while the stimulated and unstimulated parotid glands salivary flow rates remained the same with age, as published by Diaz de et al. (2014) and Al-Alimi et al. (2014). Owing to salivary diversity in composition and function, saliva can be utilized for the diagnosis of dental caries.

Dental caries occurs in a process where dissolution of crystalline mineral structure of a tooth is broken down by acids. If its cavity is left untreated it can lead to pain, infection, and tooth loss. The worldwide global index used to measure caries in epidemiological studies would be the Decayed, Missing, Filled (DMF) Index. The scoring of DMFT in subjects is based on the results of clinical examinations and the calculation of the number of decayed, missing and filled teeth. The range of DMFT score is from 0 to 28. However, the score does not specify the number of teeth that are at risk, or the number of sound teeth as stated by Radie et al. (2021).

Overall, DMFT score of the subjects was evaluated for assessing the dental caries prevalence in our study. In addition, for further unraveling the relation between dental caries prevalence with total salivary protein and flow rate, we also assessed the DMFT scores with salivary total protein level and salivary flow rate in both unstimulated and stimulated saliva in the study.

MATERIALS AND METHODS

Fifty-seven of first year dental students were selected for the study using epi-info application, with confidence level of 95%. Prior to the study, the ethics approval with reference no: REC/03/2021 (MR/103) was obtained from the Ethics Committee of Universiti Teknologi MARA. Dental records for 57 students were obtained from the Integrated Dental Record Management System (IDeRMS) of the Faculty of Dentistry. The system is widely accessible via web and also mobile device-friendly since the application is mobile responsive application. DMFT index for all subjects was extracted from IDeRMS. These selected subjects were given a written consent form denoting the willingness of the subject to voluntarily take part in this study. The oral examination

consisting of teeth charting and DMFT score were recorded and conducted with the supervision of an experienced clinician. The same clinician also verified the recorded data for all selected subjects for the study. Subjects were divided into four groups of DMFT index: DMFT 0, DMFT 1-3, DMFT 4-10 and DMFT >10.

For the protein analysis, volume of 3 to 4 mL of the unstimulated and stimulated saliva were collected from all selected subjects. For the collection of unstimulated saliva, the subject was required to sit quietly in 5 minutes duration with the head bent down to allow the saliva to pool in the floor of the mouth.

For the collection of stimulated saliva, the subject was required to rest for 5 minutes prior saliva collection. Then, paraffin wax was given to each subject for them to chew for about 5 minutes. Stimulated saliva was collected in 5 minutes duration. All collected samples were stored in a freezer at -20oC until the protein assay was performed.

The salivary flow rate of unstimulated and stimulated saliva of each subject was then calculated by using the following formula:

$$\text{Salivary flow rate (mL/min)} = \frac{\text{Volume of saliva collected (mL)}}{\text{Duration of saliva collected (min)}}$$

Saliva flow rate of unstimulated saliva can be categorized as: Normal (more than 0.25ml/min), Low (0.1-0.25ml/min) and Very low (less than 0.1 ml/min).

Saliva flow rate of stimulated saliva can be categorized as: Normal (more than 1.0 ml/min), Low (0.7-1.0 ml/min) and Very low (less than 0.7 ml/min).

Bicinchoninic Acid (BCA) protein assay was performed to measure the salivary total proteins of stimulated and unstimulated saliva for all selected subjects. The procedure was conducted according to the manufacturer's instructions. Bovine Serum Albumin (BSA) was used as the primary standard for protein assay.

0.1 mL of each standard and saliva samples were pipetted into different labelled test tubes. 0.2 mL of working reagent was then added to each tube and mixed well. All the tubes were incubated at 37oC for 30 minutes using a water bath. The absorbance of the mixture was measured using a spectrophotometer at 562nm wavelength. Reagent without sample was used as blank. The average absorbance measurement of the samples was subtracted with the blank. A range of concentrations were prepared for standard solution (0, 0.025, 0.125, 0.250, 0.500, 0.750, 1.000, 1.500, 2.000 mg/mL) and the absorbance for each concentration was also read at the same wavelength. The standard curve was used to determine the protein concentration of stimulated and unstimulated saliva for all subjects. All experiments were conducted in triplicate run. Protein concentration level categorized into: Normal (0.5-2.0 mg/mL) and High (>2.0 mg/mL).

Statistical analysis

The obtained data of the study were analysed using descriptive test by SPSS Ver. 26 Software Program (IBM, New York), and the correlation of DMFT index with various tested salivary parameters was evaluated using Spearman's correlation test. Data analysis was set significant with p value < 0.05.

RESULTS

The study subjects consisted of 57 subjects, 25% (14) are male and 75% (43) are female. The age distribution of the subjects is between 19-21 years old. The mean DMFT index with male is 3.65 and female is 3.21. It shows the male subjects had higher mean DMFT than female (Table 1).

In the DMFT index study, 11 (19%) subjects showed DMFT 0, 23 (40.6%) DMFT 1-3, 21 (36.9%) DMFT 4-10 and 2 (3.5%) for DMFT >10 respectively (Table 1).

The frequency of subjects according to the salivary total protein concentration and salivary flow rate is shown in Table 2.

Table 1: Frequency of gender, DMFT Index and mean value of the subjects

	Male	Female	Total %
Subject number	14 (25%)	43 (75%)	57 (100%)
DMFT 0	3	8	11 (19%)
DMFT 1-3	5	18	23 (40.6%)
DMFT 4-10	5	16	21 (36.9%)
DMFT >10.	1	1	2 (3.5%)
DMFT Mean Value	3.65	3.21	

Table 2: Frequency of subjects according to salivary protein concentration and flow rate

		Unstimulated saliva			Stimulated saliva		
		Male	**Female**	**Total %**	**Male**	**Female**	**Total %**
Protein concentration (mg/mL)	Normal (0.5-2mg/mL)	13 (22.80%)	38 (66.67%)	51 (89.47%)	14 (24.57%)	42 (73.68%)	56 (98.25%)
	High (>2mg/mL)	1 (1.75%)	5 (8.78%)	6 (10.53%0	0	1 (1.75%)	1 (1.75%)
Flow rate (mL/min)	Very Low	0	2 (3.51%)	2 (3.51%)	3 (5.26%)	7 (12.28%)	10 (17.54%)
	Low	4 (7.02%)	11 (19.30%)	15 (26.32%)	1 (1.75%)	1 (1.75%)	2 (3.51%)
	Normal	10 (17.55%)	30 (52.63%)	40 (70.18%)	10 (17.55%)	35 (61.40%)	45 (78.95%)

In the study of total protein concentration in the unstimulated saliva, 51 (89.47%) subjects showed normal and 6 (10.53%) of high protein concentration. Whereas in stimulated saliva, 56 (98.25%) of subjects show normal and 1 (1.75%) of high total protein concentration.

In the study of salivary flow rate, unstimulated saliva shows 40 (70.18%) normal, 15 (26.32%) low and 2 (3.51%) very low of flow rate. Whereas in stimulated saliva, shows 45 (78.95%) normal,2 (3.51%) low and 10 (17.54%) very low of flow rate.

Correlation between the overall DMFT index and protein concentration, salivary flow rate of both unstimulated saliva and stimulated saliva were evaluated by Spearman's correlation test. The correlation test done for both protein concentration and flow rate show p-value< 0.05, hence it can be concluded that significant difference exists in DMFT, salivary flow and total protein among the subjects (Table 3).

In study of total protein concentration and salivary flow rate, Spearman's coefficient r= - 0.314 (p-value 0.017) was significantly observed between unstimulated salivary flow rate and protein concentration in unstimulated saliva. In addition, for salivary flow rate study, Spearman's coefficient r= -0.244, (p-value 0.067) was slightly significantly observed between stimulated saliva and DMFT.

Table 3: The correlation between DMFT and salivary parameters

Variables	Mean	SD[1]	Spearman's correlation coefficient, r				
			DMFT	Protein concentration in un-stimulated saliva	Protein concentration in stimulated saliva	Salivary flow rate in unstimulated saliva	Salivary flow rate in stimulated saliva
DMFT	3.54	3.37	1	0.114 p-value =0.397	0.142 p-value =0.292	-0.056 p-value =0.681	-0.244 p-value =0.067*
Protein concentration in unstimulated saliva	1477.73	580.72	-	1	-	-0.314 p-value=0.017*	-
Protein concentration in stimulated saliva	1470.63	256.05	-	-	1	-	-0.164 p-value =0.224
Salivary flow rate in unstimulated saliva	0.45	0.28	-	-	-	1	-
Salivary flow rate in stimulated saliva	8.03	4.02	-	-	-	-	1

*Statistically significance

[1] SD: Standard Deviation

DISCUSSION

The total protein concentrations of subjects between genders have slightly higher mean value of protein levels in males compared to females in both stimulated and unstimulated saliva, in which corresponding to the previous study that identified mean protein concentration in females was lower than in males done by Cunha-Cruz et al. (2013). In contrast, there was a study found that women had considerably higher salivary protein levels compared to men in both caries free and caries active groups.

In our study, we found that there is a slightly significant difference in DMFT for salivary flow rate in stimulated saliva. In the stimulated salivary flow rate study, more than half of the subjects that caries-free had normal salivary rate. Concurrently, majority of the caries-risk group also had normal flow rate of saliva and very few had very low and low salivary flow rate. A similarity finding was demonstrated a low stimulated salivary flow rate was associated with increased dental caries among older adults, instead of children or adults as revealed by Pyati et al. (2018). With regards to the unstimulated salivary flow rate in our study, some of the subjects were caries-free with very low salivary flow rate, and some were low and normal flow rate. This could be because normal salivary flow rate offers a strong protection against dental caries. In the meantime, most of the study subjects with caries-risk had normal flow rate, some of them had low flow rate but none of them had very low flow rate. All the salivary parameters in our sample study were collected only in the morning, and it was avoided cascadic rhyme variant of salivary flow rate as professed by Al Alimi et al. (2014) if it was done in the morning.

In relation to the study of DMFT index with salivary stimulated and unstimulated total protein, we observed that there was no significant correlation. This is similar to a study done by Hemadi et al. (2017). specified that there was no stable association between salivary proteins and dental caries with regard to proteins phenotypes, protein molecular weight or total proteins concentration. It was also supported by other study done by Pyati et al. (2018), that there is no significant difference of mean protein levels between caries free group and early childhood caries patients. The reasons might be due to the different roles of salivary proteins in oral cavity, for example adhesins and agglutinins increase the establishments of microorganisms while the other antimicrobial and pH modulating proteins act as defensive mechanisms. A different study also mentioned that there is no significant difference in concentration of total salivary proteins between groups with or without early childhood caries owing to the dissimilar structure levels and function of saliva. In contrast, one study mentioned higher levels of total salivary proteins were found in the caries free group compared to the caries group, shared in a study by Hemadi et al. (2017). In addition, a systematic review reported that the majority of the studies have statistically significant differences between individuals with or without caries experience.22

In general, the relationship between total salivary protein, salivary flow rare and DMFT score has been studied by several researchers, but the results are varied. It is notable that many factors could affect the differentiation between each study such as sample size, age, gender and type of saliva in the study of Hegde et al. (2019). Thus, larger population size with different cohort in assessing the dental caries prevalence with salivary parameters is suggested for future study.

In conclusion, our study concluded that the findings revealed the caries risk is associated with salivary flow rate. Thus, the findings may serve as reference values for the growing interest in saliva as a caries diagnostic tool.

CONFLICT OF INTERESTS

The authors declare to have no conflict of interest.

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