

A Salivary α -Amylase Level in Relation to the Oral Health Parameters among Children in Baghdad City

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ABSTRACT

Background: Saliva is a specific bio-fluid with important biomarkers. Analyzing any alternation in these markers could give valuable information, in relation to oral health status parameters. The aim of this study was to investigate the level of α -amylase in unstimulated whole saliva of healthy, primary school children in relation to some oral health parameters.

Materials and Methods: A questionnaires consisted of demography and medical histories of participants were filled by children families. Saliva samples were collected for 5- minutes between 9:00 -11:00 AM from 114 healthy students aged 6-13 years, divided into four age groups. Flow- rate, Plaque and Gingival Index were assessed and dentition status was investigated by DMFT/dmft using WHO criteria. Salivary amylase was analyzed in unite per litter, using quantitative colorimetric amylase determination at 585nm.

Results: A significant positive correlation was found between age and salivary flow-rate, ($r=0.362$, $P < 0.001$). Salivary α -amylase concentration increased significantly with age ($P < 0.001$). For each one year there is an increase in age, amylase level is expected to increase by 5.2 U/L. A male gender is expected to reduce salivary α -amylase level by 10.6 U/L compared to female, however the effect was not significant. Gingival index was positively, although non-significantly associated with salivary α -amylase concentration. DMFT showed a significant weak positive linear correlation with salivary amylase level ($r=0.309$, $P < 0.001$), while deciduous teeth decay experience and plaque index were significantly and negatively associated with salivary amylase.

Conclusion: Results emphasize the importance of salivary amylase, as a non-invasive biomarker in regulating oral and dental health status in children.

Key words: Salivary α -amylase, unstimulated whole saliva, oral health parameters. (J Bagh Coll Dentistry 2016; 28(2):40-46).

INTRODUCTION

Alpha-amylase is one of the principal salivary proteins, accounting for 10–20% of the total protein content ⁽¹⁾. The main function of salivary alpha-amylase is the enzymatic digestion of carbohydrates through hydrolysis of starch to glucose and maltose with optimum pH of 6.7-7.0⁽²⁾. It is known for its role in the breakdown of large insoluble starch molecules into smaller, soluble molecules. Additionally, salivary alpha-amylase has been suggested to inhibit the adherence and growth of bacteria to prevent bacterial attachment to oral surfaces and to enable bacterial clearance from the mouth ⁽³⁾, so it is important for the mucosal immunity in the oral cavity ^(4,5).

Alpha-amylase is secreted by the salivary gland and pancreas and so present in saliva and serum. In the salivary gland, alpha-amylase is synthesized and secreted by highly differentiated epithelial acinar cells which make up more than 80% of the cells in the major salivary glands, mostly of the parotid glands ⁽²⁾.

Salivary alpha-amylase, secreted following activation of beta-adrenergic receptors, so it may measure the endogenous sympathetic activity. Alpha- amylase enzyme produced mainly by serous cells of the parotid gland followed by sublingual, submaxillary, and minor glands. Typically, the concentration of amylase in human saliva ranges from 0.04 - 0.4 mg/ml ⁽⁶⁾ increases during food consumption and with stress ⁽⁷⁾.

Several lines of evidence showed that alpha-amylase has multifunction in the oral cavity. The initial enzymatic digestion of dietary starch begins in the oral cavity with the release of maltose and maltodextrin, providing an abundant source of carbohydrate for oral bacterial nutrition. Secondly, in addition to its hydrolytic activity, alpha-amylase binds to a selected group of oral streptococci, that may contribute to bacterial clearance and nutrition ^(8,9).

The binding of alpha- amylase to bacteria in solution may be considered protective if it leads to bacterial clearance from the oral cavity ⁽⁵⁾. The fact that alpha -amylase binds to teeth as a constituent of enamel pellicle play a role in modulating the adhesion of bacterial species to the teeth ⁽⁸⁾.

It is well recognized that dental plaque is closely related to the most common oral diseases, dental caries and periodontal disease ⁽¹⁰⁾. Formation of dental plaque is a complex process

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includes the interaction between streptococci and the salivary protein alpha-amylase^(11,12).

In brief, salivary amylase contributes in at least three vital roles affecting biofilm: 1) hydrolysis of dietary starch, 2) binding to the tooth surface, and 3) binding to oral streptococci⁽¹³⁾.

Considering age and gender, similar to other body organs, it has been clearly established that synthesis and secretion of enzymes in unstimulated saliva decrease with age^(14,15). This has been supported by a study conducted by Kalipatnapu et al. who showed that increase in salivary protein and amylase in both males and females up through middle age and then the concentration of these constituents remain unaffected through the rest of adult life⁽¹⁵⁾. In the same study, males secrete more saliva than females, accordingly, they synthesize and secrete greater amounts of salivary protein and amylase compared to females.

This study aimed to investigate alpha- amylase level in unstimulated whole saliva of primary school children in relation to some oral health parameters in this group of children.

MATERIALS AND METHODS

Participants

One hundred fourteen primary school students took part in this study. They were recruited from Ashoor- primary school in Baghdad/Iraq. One class from each year of study was invited to take part in this study; six classes from year one to year six.

The study was approved by the Ethics Committee of Oral Diagnosis Department in the College of Dentistry –University of Baghdad.

Questionnaire distributed

Necessary permission was taken from school authorities and written informed consents were taken from the parents/guardians before the start of the study. A description about the purpose and aim of the study was performed for both school authorities and families of participants.

The study was carried out using a structured questionnaire that was distributed in Arabic language and sent to the families of the school children; completed by the parents /guardians and return back to the school. These questionnaires consisted of three parts; the first part was related to the demography of children regarding name, age, gender and year of study. The second part involved clinical oral examination that carried out under natural light using disposable plane mouth

mirrors and WHO dental explorers for diagnosis of dental caries. Oral hygiene and gingival health status was determined by plaque index (PI)⁽¹⁶⁾, gingival index (GI)⁽¹⁷⁾ and calculus index (CI)⁽¹⁸⁾ by using periodontal probe. Dentition status was also investigated by DMFT, and dmft using WHO criteria⁽¹⁹⁾. Oral examination was performed by the same examiner. The third part of the questionnaire considered the child medical history. The questions were mainly closed –ended rather than open questions, thus to avoid the misunderstanding.

Inclusion criteria of the current study were children with informed consent from the parent or guardian. Exclusion criteria were children having difficulty in opening the mouth, children who had taken antibiotics in the last month, those with systemic disease and children with orthodontic appliances.

Method of saliva collection:

Saliva collection was scheduled after the clinical examination. Saliva was collected from all participants under the same conditions⁽²⁰⁾. The children were supervised and instructed to be comfortably seated with their head tilted slightly forward. Additionally, they were instructed to swallow any saliva in their mouth, immediately before the collection started.

In brief, saliva was allowed to accumulate in the floor of the mouth and to expectorate all saliva formed over 5 minutes period into the graduated sterile test tube. The saliva samples of all the participants were identified by a code number during the period of sample collection and processing. After the disappearance of the salivary froth, the salivary flow rate was estimated in millilitres per minutes. Samples were stored at -80°C, until analysed. Sampling sessions were limited to the hours between 9:00 and 11:00 AM to minimize the effect of diurnal variations.

Quantitative Colorimetric Amylase Determination at 585nm was used to estimate the concentration of salivary amylase (U/L)⁽²¹⁾. EnzyChrom™ α -Amylase Assay Kit (ECAM-100).

Statistical analysis

Statistical analysis was performed with SPSS version 19.0. Descriptive statistical analysis, student T-test, analysis of variance (ANOVA) and linear and multiple linear correlation were used. A p-value of less than 0.05 was considered to indicate statistical significance.

RESULTS

Age, Flow Rate and Salivary Amylase Level

One hundred fourteen primary school students were enrolled in this study, 64 (56%) were females and 50 (44%) were males. The age ranged from 6-13 years with a mean of 9.1 years. As shown in table 1, students were divided into four age groups: 6-7, 8-9, 10-11 and 12-13 years. Mean age of the female students was 9.2 years and that of male was 8.9 years.

A significant correlation was observed between age and salivary flow rate ($r=0.395$; $P < 0.001$), higher salivary secretion produced with increased age; table 1 and figure 1.

The age of children showed a significant positive linear correlation with salivary amylase level, as shown in table 2.

Overall, the unstimulated salivary flow rate for the primary school children ranged from 0.02 ml/min for the lowest and 1.1 ml/min for the highest rate. Flow rate for female students ranged

from 0.02 -1.1 ml/min and for male from 0.15 to 0.09 ml/min, with male students produced higher mean salivary flow rate than female (0.341 vs. 0.320 ml/min), however the difference was non-significant.

As shown in table 1, under unstimulated conditions, the mean salivary amylase is lowest in the youngest age group (6-7) years with a mean of 270.6 U/L, and increased with advancing age to reach a maximum of 309.7 U/L for the oldest age group (12-13 years). The difference observed in salivary amylase between age group was highly significant ($p < 0.001$).

To assess the net and independent association of a set of explanatory variables on salivary amylase concentration as the dependent variable, a multiple linear regression model was used. The explanatory variables include: plaque index, gingival index, salivary flow rate, decayed deciduous teeth surfaces, decayed permanent teeth surfaces, age and gender.

Table 1: Age and oral health status parameters in relation to salivary alpha-amylase

Indices	Description	Salivary Amylase (U/L)				
		Range	Mean	SE	N	P
Plaque index	Good (0.1 -1)	(287.9 – 334.9)	305.8	14.7	3	0.72 F=0.336
	Fair (1.1-2)	(187.2 – 373.0)	287.2	4.1	63	
	Poor (2.1-3)	(133.5 – 371.5)	287.5	6.6	48	
Gingival index	Mild (0.1 -1)	(150.1 – 373.0)	281.9	6.5	40	0.22 F=1.508
	Moderate (1.1-2)	(133.5 – 371.5)	291.1	4.3	74	
Age group (years)	6-7	(133.5 – 358.7)	270.6	7.6	29	<0.001 F=5.933
	8-9	(150.1 – 373.0)	281.2	6.1	35	
	10-11	(212.5 – 371.5)	300.6	5.8	34	
	12-13	(272.3 – 367.1)	309.7	7.1	16	
Salivary flow Rate categories	First (lowest) tertile (≤ 1.00)	(150.1 – 367.1)	281.0	5.1	56	0.14 F=1.982
	Second (average) tertile (1.01 – 2.00)	(133.5 – 373.0)	297.3	8.5	28	
	Third (highest) tertile (2.01+)	(240.2 – 371.5)	293.0	5.7	30	
dmft	First (lowest) tertile (≤ 2)	(235.7 – 371.5)	298.3	4.6	43	0.03 F=3.551
	Second (average) tertile (3 – 7)	(187.2 – 373.0)	286.6	6.2	37	
	Third (highest) tertile (8+)	(133.5 – 358.7)	275.5	7.7	34	
DMFT	First (lowest) tertile (0)	(133.5 – 373.0)	281.4	4.6	66	0.07 F=2.699
	Second (average) tertile (1)	(227.5 – 345.3)	286.8	9.4	11	
	Third (highest) tertile (2+)	(150.1 – 367.1)	299.3	6.4	37	

As shown in table 3, among the tested explanatory variables, only the age showed a statistically significant positive association with salivary amylase level after controlling for the

remaining explanatory variables included in the model. For each one year increase in age, the salivary amylase concentration is expected to increase by an average of 5.2 units.

Table 2: Linear correlation coefficient test

Variables	Salivary Amylase
Salivary flow rate	r=0.193 P=0.044
Plaque index	r=0.078 P=0.41
Gingival index	r=0.194 P=0.04
Decay primary teeth (dt)	r=-0.184 P=0.05
Missing primary teeth (mt)	r=-0.026 P=0.78
Filling primary teeth (ft)	r=-0.005 P=0.96
dmft	r=-0.186 P=0.047
Decay permanent teeth (DT)	r=0.300 P=0.001
Missing permanent teeth (MT)	r=0.186 P=0.048
Filling permanent teeth (FT)	r=0.114 P=0.23
DMFT	r=0.309 P=0.001
Age	r=0.395 P=0.001

Table 3: Multiple linear regression model with salivary amylase concentration as the dependent (response) variable and selected independent variables

	Partial regression coefficient	P	Standardized regression coefficient
(Constant)	224.5	<0.001	
Age (years)	5.2	0.02	0.27
Male gender compared to female	-10.6	0.15	-0.14
Gingival index	36.5	0.19	0.13
Plaque index	-10.0	0.35	-0.09
Decayed primary teeth (dt)	-0.48	0.38	-0.09
DT	1.9	0.41	0.09
Salivary flow rate	0.27	0.94	0.01

$R^2=0.21$, P (Model) =0.005

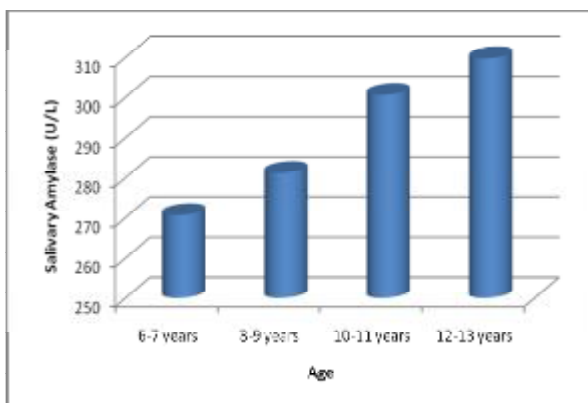


Figure 1: Age group in relation to mean salivary amylase concentration.

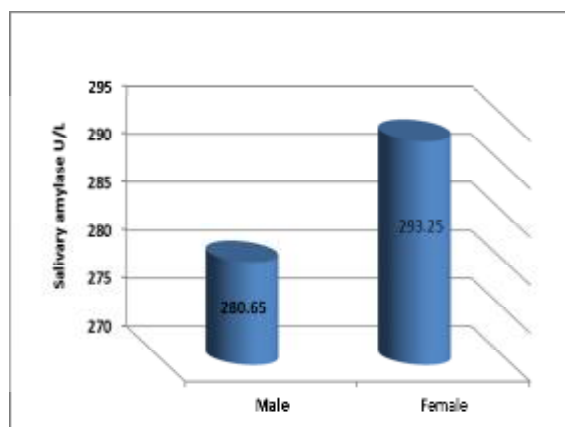


Figure 2: Gender and mean salivary amylase level

Gender and Salivary Amylase Level

Considering gender, figure 2 shows that male students showed lower mean salivary amylase level compared to female students, however the difference was not significant. Multiple linear regression analysis showed that being a male is

expected to reduce salivary amylase by a mean of 10.6 U/L compared to female after adjusting (controlling) for the possible confounder effect of the remaining explanatory variable included in the model. The effect of gender, however failed to reach the level of statistical significance.

Plaque index (PI) and Salivary Amylase Level

As shown in table 1, plaque index scores were categorized into good, fair and poor scores. The majority of the students (55%) were found with fair (moderate) scores, followed with severe (42%), and only 3% of were with mild scores of PI. Although, mean salivary amylase level was higher in children with mild PI scores, it showed no statistically significant differences between different categories. In relation to gender both male and female showed the same mean plaque index score (1.943 ± 0.380).

Gingival index and Salivary Amylase Level

Gingival index was categorized into mild and moderate score, with the majority of study groups were within the moderate scores 64% (73/114) as shown in figure 3.

There was a significant positive linear correlation between gingival index and salivary amylase concentration ($P= 0.04$). Children with moderate gingivitis observed with higher mean of salivary amylase levels (291.1 U/L) compared to those with mild gingivitis (281.9 U/L), however the mean differences were unable to reach the level of statistical significance.

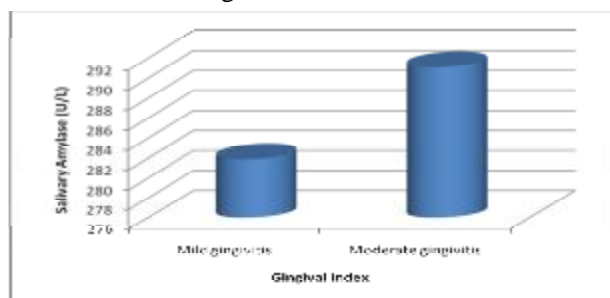


Figure 3: Gingival score index in relation to mean salivary amylase level

Caries experience and salivary amylase level

Decayed-Missing-Filled-Tooth for primary and permanent teeth index scores were divided into three groups, the lowest, average and the highest tertile as seen table 1.

DMFT showed a statistically significant weak positive linear correlation with salivary amylase level ($r=0.309$, $P<0.001$). The mean salivary amylase was smallest among students with 1st lowest tertile DMFT scores (281.4 U/L) and increased with increased DMFT index scores to reach a maximum of 299.3 U/L among students in the third (highest) tertile DMFT group; figure 4. However, the difference observed in the mean salivary amylase between DMFT categories failed to reach the level of statistical significance, possibly because of small sample size.

Considering DT, multiple linear regression analysis showed that for each 1 unite increase in DT, the mean salivary amylase level is expected to increase by amount of 1.9 U/L. This effect however was not significant.

Regarding dmft index, in this study, results showed statistically significant relation with mean salivary amylase level.

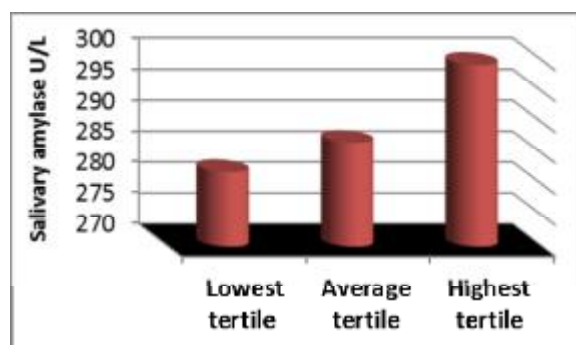


Figure 4: Caries experience (DMFT) in relation to salivary α -amylase level

DISCUSSION

Establishing important data information was an important aim of this study as it is vital for children oral health status management.

Variables that could influence salivary flow rate were minimized; all children were from the same economic status, none of them were taking any medication or presented with any systemic disease.

Considering flow rate, there was a significant correlation between age and both salivary flow rate and salivary amylase level. This is consistent with the observation from previous studies that salivary flow- rate, protein and enzyme contents in saliva increased with age. Salivary flow rate increases with age in children and adolescent populations⁽²²⁻²⁵⁾ although others have reported controversial findings^(26, 27).

In this study, male students produced higher mean salivary flow rate than female, however the difference was non-significant. This finding agrees with previous clinical study that showed a higher saliva output of males than females even in children populations⁽²²⁻²⁵⁾.

This may be explained on a base of hormonal alterations that have been suggested to influence salivary flow rate⁽²⁵⁾.

Multiple linear regression analysis showed that male gender reduce salivary amylase by more than ten- times compared to female after adjusting other confounders, however non-significant. The non-significant relation may be due to the small sample size. This is in contrast to the previous study which found a higher level of salivary alpha amylase in male participants⁽²⁸⁾. The controversial finding may

be due to different age group and socioeconomic status of the study population.

Regarding age, for each one- year increase in age, salivary amylase concentration is expected to increase by 5.2 units. This is supported by previous studies that found the level of salivary alpha amylase activity increased along with age^(28,29).

Saliva plays a vital role on caries development through, mechanical cleansing, dilution of substances in the oral cavity, de- and -re-mineralization of dental enamel, pellicle formation, antimicrobial action and buffering of acids produced by biofilm and diets^(30,31).

Studies addressed the relationship between salivary alpha-amylase and dental caries are few, and the results are in consistent. Some research supported the correlation between alpha amylase and dental caries while the others did not.

In this study, the relationship between salivary alpha-amylase and DMFT scores was assessed, an increase in amylase level was associated with increased DMFT index scores to reach the maximum level among primary students in the third (highest) tertile DMFT group.

Multiple linear regression analysis showed that for each one- unite increase in Decay tooth (DT), there will be approximately two-fold increase in salivary amylase level. This finding supported by Scannapieco et al., study who found that alpha-amylase can promote catalyzing the hydrolysis of dietary starch via binding on the surface of cariogenic bacteria⁽³²⁾. Consequently, plaque containing amylase-binding bacteria results in concentration of salivary amylase within the plaque matrix providing more glucose in proximity to the tooth surface. Finally, this plaque plays a significant cariogenic role in the presence of starchy foods⁽³³⁾.

On the other hand, the present study is in consistent with de Farias et al. study who did not confirm the relationship between alpha-amylase and dental caries⁽³⁴⁾. Additional research with larger sample sizes is required to confirm these findings.

There is sparse literature regarding the relationship between salivary amylase and gingivitis. In the present study, children with higher scores of gingivitis showed the highest levels of salivary amylase. This finding is in accordance with other studies which showed an increased concentration of salivary amylase in patients with gingivitis⁽³⁵⁻³⁷⁾.

In addition to its digestive action, α - amylase exhibits inhibitory activity against various microorganisms⁽³⁸⁾ and thus may contribute in the oral defence mechanism⁽³⁹⁾. Thus, salivary amylase may be regarded as an important biochemical parameter of inflammation of the periodontium. The higher levels of amylase may be due to the

response of salivary glands to inflammatory diseases like gingivitis leading to increase synthesis and secretion of certain acinar proteins (α -amylase) to enhance the oral defense mechanism^(39, 40). This may indicate the important of salivary amylase as a defence molecule for the innate immunity in the oral cavity. Moreover, it has been suggested that the increased levels of amylase may be due to plasma proteins leakage into saliva due to inflammation⁽⁴¹⁾.

As conclusion;

1. The results of the present study emphasize the importance of salivary amylase, as a non-invasive biomarker in regulating oral and dental health.
2. Future research with larger samples is required to support these findings.
3. The inconsistency between the results may be explained in the bases that saliva is composed mainly of water and several other components; study the effect of some constituents leaving the others may lead to different results because of the confounding effect of the other components⁽⁴²⁾.

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