

## Evaluation of salivary and serum alpha amylase level in dental caries of adolescence

Avaliação do nível de alfa-amilase salivar e sérica na cárie dentária da adolescência

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### ABSTRACT

**Objective:** Previous studies suggested a significant relationship between alpha- amylase and caries formation. This study was implemented in order to investigate the interrelation between level of salivary and serum alpha- amylase, and dental caries. **Methods:** In this cross-sectional study, un-stimulated whole saliva and serum samples were collected from 118 high school students who were divided into four groups: caries free females (N = 28), caries active females (N = 35), caries free males (N = 28) and caries active males (N = 27). Mean levels of salivary and serum alpha-amylase was evaluated by spectrophotometric methods to assay enzyme kinetics. Data were analyzed using student's t-tests and chi-square tests. **Results:** Salivary alpha-amylase was significantly higher in caries active groups as compared to caries free groups (P = 0.002). The mean salivary alpha -amylase was significantly higher in caries active males (P = 0.002). Also, serum alpha -amylase was significantly higher in caries active groups as compared to caries free groups (P = 0.001). In both sexes the mean serum alpha- amylase levels were statistically significant higher in caries active compared to caries free groups (P = 0.02 and 0.01 respectively). **Conclusion:** A significant association was found between salivary and serum alpha- amylase and dental caries in adolescents. More research to demonstrate the real relation between alpha amylase and dental caries is recommended.

### KEYWORDS

Alpha-amylase; Dental caries; Saliva; Serum.

### RESUMO

**Objetivo:** Alguns estudos anteriores sugeriram uma relação significativa entre a alfa-amilase e a formação de cárie. Este estudo foi realizado com o objetivo de investigar a interrelação entre o nível de alfa-amilase na saliva e no soro e a cárie. **Material e Métodos:** Neste estudo transversal, amostras de saliva não-estimulada e soro foram coletadas de 118 estudantes do ensino médio que foram divididos em quatro grupos: mulheres livres de cárie (n = 28), mulheres com cárie ativa (n = 35), homens livres de cárie (n = 28) e homens com cárie ativa (n = 27). O nível de alfa-amilase da saliva e soro foi avaliado por espectrofotometria para ensaios de cinética enzimática. Os dados foram analisados pelos testes "t" e Qui-quadrado. **Resultados:** A alfa-amilase salivar foi significativamente maior nos grupos com cárie ativa em comparação com os grupos livres de cárie (p = 0,002). A alfa amilase salivar foi significativamente maior nos homens com cáries ativa (p = 0,002). Além disso, a alfa amilase sérica foi significativamente maior nos grupos de cárie ativa, em comparação com os grupos livres de cárie (p = 0,001). Em ambos os sexos a média da alfa-amilase sérica foi estatisticamente superior nos grupos de cárie ativa, em comparação com livres de cárie (p = 0,02 e 0,01, respectivamente). **Conclusão:** Foi encontrada uma associação significativa entre a alfa-amilase salivar e sérica em indivíduos com a cárie dentária na adolescência. São recomendadas mais investigações para demonstrar real relação entre os níveis de alfa-amilase e cárie dentária.

### PALAVRAS-CHAVE

Alfa-Amilases; Cárie dentária; Saliva; Soro.

## INTRODUCTION

Dental caries is an infectious, communicable disease resulting from demineralization and destruction of tooth structure by acid-forming bacteria [1]. Tooth decay is one of the most prevalent diseases among 5 to 17 year-old American children [2]. According to the WHO, tooth caries are considered as the fourth world plague, after cancers, cardiovascular disease, and HIV infection [2,3].

Saliva is a biological fluid that represents all the ions usually present in body fluids, and is able to buffer acids that it is essential for maintaining pH values in the oral environment. Moreover, saliva has been identified as a putative indicator for dental caries [1]. The salivary components, such as proteins and enzymes, (in addition to their protective function), have important roles through the various interactions with oral bacteria, such as antibacterial activities, or providing receptors for bacterial adhesion [4]. Salivary alpha-amylase is an abundant calcium-containing metalloenzyme produced by serous cells of the parotid and other salivary glands [5]. This enzyme plays an important role in binding of *Streptococcus* and other co-inhabitants of dental biofilm [6,7]. Salivary alpha-amylase also hydrolyzes starch to glucose and maltose; giving rise to products that are transformed into acids leading to dental caries [5,8]. Although some previous studies suggested a significant relationship between alpha- amylase, dental plaque, and caries formation [8-10] others reported a completely opposite concept [2,3,11]. They believed this enzyme could help to provide dietary starch granules that are swallowed, and plays role in the clearance of bacteria from the oral cavity, so may have an anticariogenic effect [2, 3]. Considering the inconclusive results for these alternate hypotheses [11], the aim of this study was to evaluate the interrelation between the level of salivary and serum alpha- amylase and dental caries.

## MATERIALS AND METHODSE

### Ethics

This descriptive cross-sectional study was approved by the ethics committee of Hamadan University of Medical Sciences, Hamedan, Iran. Informed consent was obtained from the parents of each student. This study was carried out from 2012 to 2013 in Hamadan city, west of Iran.

### Participants

A total of 118 high school students between 15-19 years old (including 63 females and 55 males) were selected and divided into four groups: caries free (CF) females (N = 28), caries active (CA) females (N = 35), caries free males (N = 28) and caries active males (N = 27). Participants with a history of any systemic diseases, regular users of medications within the past 3 months, as well as those with poor oral hygiene or periodontal disease were excluded.

### Clinical examination of Caries

A single examiner carried out all clinical examinations. The ICDAS (International Caries Detection and Assessment System) [12] criteria were employed: 0) sound; 1) first visual change in enamel; 2) distinct visual change in enamel when wet; 3) localized enamel breakdown (without clinical visual signs of dentine involvement); 4) underlying dark shadow from dentine; 5) distinct cavity with visible dentine; and 6) extensive distinct cavity with visible dentine. A score of 0 was considered normal. A score of 2 was considered caries restricted to the enamel without dentine involvement. Teeth with cavity formation were considered as those with scores of 3, as rupture of the structure of the enamel surface is seen beginning at this point. Scores of 5 and 6 were considered severe dental caries, as these scores determine obvious cavitation extending through the dentine. Dental caries was recorded when at least five teeth received code 5 and 6. The dental examination was performed in a dental chair, using a dental mirror and an explorer without radiographic examination. CF

group did not have any caries and filling as well as sign and symptom of teeth sensitivity (score of 0). There was no statistical difference between groups considering the student's age and gender ( $p > 0.05$ ).

#### Saliva collection

Whole unstimulated saliva was collected over a time span of 5 min (10) between 8 to 11am. We advised the students to brush their teeth and do not use any oral stimulation such as eating and drinking for 90 min prior to collection of saliva, then we asked them to sit on a chair and lean their head forward over the test tube. Whole saliva samples were obtained by expectorating into polypropylene tubes. The saliva samples were kept at 4°C and were transferred to the laboratory up to 20 min and stored at -20°C for final measurement of alpha-amylase level.

#### Blood collection

Blood samples were obtained by venous arm puncture and collected in heparinized tubes.

#### Sialochemical analysis

In the laboratory, saliva samples were centrifuged (KD2-TDSA, Nantong Hailun Bio-medical Apparatus Manufacturing C., Ltd., Haimen city, Jiangsu, China) for 3 - 5 min in order to acquire pure saliva. The salivary alpha-amylase activity was assayed by a biochemical kit (EPS-G7, Pars Azmoon Co, Karaj, Iran) and a spectrophotometer (6300, Jenway, Staffordshire, UK) at a wavelength of 590 nm. In this method, the reaction of alpha amylase on a chromogenic substrate produced a coloured solution of chloro-p-nitrophenol, and its darkness was proportional to the level of enzyme activity [11]. Serum alpha amylase activity was also measured similarly, using commercially available kits (Pars-Azmoon Co., Iran) that used to measure amylase activity [13, 14]

#### Statistical analysis

Data were analyzed using student's t-test and chi-square test with Stata.11 software. The values are expressed as mean  $\pm$  SD. A P value of  $<0.05$  was considered statistically significant.

## RESULTS

The comparison between salivary alpha-amylase levels in different groups is shown in table 1. Salivary alpha-amylase was significantly higher in caries active groups ( $P = 0.002$ ). However, mean salivary alpha- amylase levels were not significantly higher in females than males ( $P = 0.71$ ). Although the mean salivary alpha -amylase was significantly higher in caries active males compared to caries free ( $P = 0.002$ ), it was not significant in caries active females ( $P = 0.28$ ).

The comparison between serum alpha-amylase levels in different groups is shown in table 2. Serum alpha -amylase was significantly higher in caries active group as compared to caries free group ( $P = 0.001$ ), but mean Serum alpha -amylase levels were not significantly higher in males than females ( $P = 0.42$ ); and the mean serum alpha -amylase was significantly higher in caries active males ( $P = 0.02$ ), just like the salivary amylase result. On the contrary, the mean serum alpha- amylase was statistically significantly higher in caries active females ( $P = 0.01$ ).

**Table 1** - Comparison of salivary alpha- amylase in patients with dental caries, according to gender and caries status

Variable	Mean Saliva Amylase	Std. Err	[95% Conf.	Interval]	P value
Male	152.61	1.80	149.00	156.21	P=0.71
Female	153.43	1.39	150.65	156.21	
Caries free	149.56	1.69	146.16	152.96	P=0.002*
Caries active	156.19	1.36	153.47	158.92	
Caries free male	147.35	2.28	142.68	152.02	P=0.002*
Caries active male	158.06	2.42	153.09	163.03	
Caries free female	151.77	2.48	146.68	156.86	P=0.28
Caries active female	154.76	1.52	151.67	157.85	
Caries free male	147.35	2.28	142.68	152.02	P=0.19
Caries free female	151.77	2.48	146.68	156.86	
Caries active male	158.06	2.42	153.09	163.03	P=0.23
Caries active female	154.76	1.52	151.67	157.85	

**Table 2** - The level of serum alpha -amylase in patients with dental caries, according to gender and caries status

Variable	Number	Mean serum Amylase	SD	Std. Err	[95% Conf.	Interval]	P value
Male	53	244.53	35.28	4.85	234.81	254.26	p=0.42
Female	53	238.59	40.99	5.63	227.29	249.89	
Caries free	49	228.89	38.07	5.44	217.96	239.83	p=0.001*
Caries active	57	252.45	35.05	4.64	243.15	261.75	
Caries free male	26	233.72	30.05	5.89	221.58	245.86	p=0.02*
Caries active male	27	254.94	37.30	7.18	240.19	269.70	
Caries free female	23	223.43	45.58	9.50	203.72	243.14	p=0.01*
Caries active female	30	250.21	33.27	6.09	237.74	262.67	
Caries free male	26	233.72	30.05	5.89	221.58	245.86	p=0.35
Caries free female	23	223.43	45.58	9.50	203.72	243.14	
Caries active male	27	254.94	37.30	7.18	240.19	269.70	p=0.61
Caries active female	30	250.21	33.27	6.09	237.74	262.67	

## DISCUSSION

Previous studies indicated at least three important functions for alpha -amylase: hydrolysis of starch, bindings to oral Streptococci, and binding to the tooth surface [12,15]. Binding of a-amylase to bacteria and teeth has important implications for dental plaque along with caries formation [1,16]

In this study, the effect of confounding variables such as increased stress, exercise, smoking; and caffeine was limited in precipitations due to the strong impact of these factors on salivary alpha- amylase concentration [12,17].

The results of this study demonstrate that salivary alpha- amylase level were significantly higher in caries active cases as compared to caries free group.

Previous studies were inconsistent to finding the relationship between salivary alpha -amylase and dental caries. Some studies supported this correlation in line with our findings [1,11,18-25]. For instance, Singh and et al reported that the amylase concentration was higher for caries active group as compared to caries-free. They showed a direct relationship between amylase and DMFS [1]. According to Volker et al [18], more acid was produced due to processing of the starch by salivary alpha- amylase. Also, Douglas et al. [19] reported that bacteria coated with alpha-amylase could produce acid by fermentation of starch. According to Toors [20] and Douglas [21], *Streptococcus mutans* alone could not ferment starch, whereas bacteria coated with alpha- amylase in dental plaque produce acids following starch exposure. In fact, salivary alpha-amylase facilitates dietary starch hydrolysis to provide glucose with subsequent acid formation leading to tooth demineralization and dental caries [11,15].

Dodds et al. indicated that alpha- amylase activity was higher in dental plaque in cases on a high sucrose diet than from subject on a low sucrose diet [22], on the other hand bacterial plaque having high level of alpha- amylase may provide more glucose from hydrolysis of starch in proximity of teeth [11].

Fiehn et al reported the level of alpha -amylase activity was significantly different between caries inactive and high caries active subjects [23]. Alpha -amylase adsorb to tooth enamel and found in acquired enamel pellicle also selectively bind to several oral commensal *Streptococci* species and facilitated the adhesion of different bacteria species to each other [12,27]. Thus, interaction between alpha- amylase bacteria and tooth contribute to the establishment

of dental plaque and subsequently promote dental caries [12,15, 24, 25].

On the other hand, other researchers did not support this role of alpha- amylase in dental caries [11,26-32]. For example; Scannapieco and colleagues reported *Streptococcus mutans*, as an important cariogenic organism, could not bind to salivary alpha-amylase [11]. Some in vivo studies also do not support the role of alpha- amylase *Streptococcus* interaction in biofilm formation [26]. Alpha- amylase might provide two bacterial interactions by extra cellular protein network and contribute as a bridge between kinds of *Streptococcus* species in establishment of dental plaque and caries [6,33].

Moreover, in contrast to this research, some previous studies on early childhood caries showed no strong correlation between dental caries and salivary total protein concentration and amylase activity [30,27,28]. Also, Mojarad et al revealed a significant but inverse correlation between salivary alpha-amylase level and early childhood caries [31]. They believed alpha -amylase probably has a protective role against early childhood caries. However, its efficacy might be decreased when caries is already established, or dental caries might exacerbate the salivary alpha -amylase level [31,34].

We studied the role of salivary alpha-amylase in tooth caries of young children not in early childhood caries. Arhakis and et al reported an influence of age on salivary alpha-amylase activity. In newborns the salivary alpha-amylase activity detected is very low, but increases to the adult levels within the first 3 years. Over the adulthood until older ages the salivary alpha-amylase activity seems to remain unchanged. Then higher salivary alpha-amylase concentrations it was seen in elderly participants compared to young adults. So it seems the effects of salivary alpha-amylase on dental caries can be different in different ages [32].

In the present research, the mean salivary alpha- amylase level was not significantly different between males and females. Similar findings were reported in a study of Bos and co-workers [35]. They did not observe any difference in alpha-amylase levels at the different time points across the day between males and females. According to our results, in male and female groups, salivary alpha- amylase level was higher in caries active groups rather than caries free, but the difference was not statistically significant in female groups. This was in agreement with the main result of our study and emphasized the positive association between the level of salivary alpha- amylase and dental caries.

We also compared the levels of serum alpha- amylase between active and inactive caries groups. The result showed serum alpha-amylase was significantly higher in caries active groups. So, dental caries is significantly associated with increased mean levels of both saliva and serum alpha- amylase. Similar findings were observed in saliva, the level of serum alpha- amylase was not significantly different between females and males, and no significant difference was observed between genders in caries free and caries active groups. In both genders, caries active cases tended to show higher levels of serum alpha-amylase than caries free cases. According to results, caries could affect salivary and serum alpha amylase level. Evaluation of salivary and serum alpha amylase could be helpful tools for determination of high risk individuals, caries prevention and treatment.

Serum amylase was determined for diagnostic purposes, such as acute pancreatitis [29]. However, salivary amylase has different origins and are produced by salivary glands [36]. Although our study showed that serum alpha amylase are increased in caries active groups, finding a correlation between serum and salivary alpha—amylase, and serum alpha—amylase and dental caries, more studies are needed.

## CONCLUSION

The results of this study demonstrated a significant association between salivary and serum alpha- amylase in adolescents with dental caries. More studies are necessary with large sample sizes, in order to understand the real role of dental caries on saliva and overall serum alpha - amylase levels.

## ACKNOWLEDGEMENT

This article was part of postgraduate student thesis and was supported by Hamadan University of Medical Sciences. The author would like to thank prof. Marc Tennant and Prof. Esti Kruger of the University of Western Australia for assistant and advice with this project.

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Date submitted: 2015 Dec 15

Accept submission: 2016 Apr 24