

ANALYSIS OF SALIVARY LEVEL OF ALPHA-AMYLASE AS A RISK FACTOR FOR DENTAL CARIES

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Abstract: The biologic properties of salivary alpha-amylase are a subject of intense debate, regarding its use as a biomarker for local and systemic diseases, including caries lesions. The purpose of our study was to measure the correlation between the salivary level of alpha-amylase and the incidence of caries. A total of 142 children were included in four study groups, based on specific inclusion/exclusion criteria. From each patient, 10 ml of saliva was collected and examined with a spectrophotometer in order to determine the level of salivary alpha-amylase. The data were statistically analyzed with the Graph Pad Prism 7.03 and Mann-Whitney test, a value of $p < 0.05$ being considered statistically significant. Our results showed that caries active children had higher levels of salivary enzyme ($p < 0.001$). Therefore, we concluded that salivary alpha-amylase can be considered a biomarker for the prognosis of dental caries development, offering new perspectives for preventive dentistry.

INTRODUCTION

Salivary alpha-amylase also known as ptyalin is a protein enzyme that hydrolyses the alpha bonds from large insoluble polysaccharides as starch into soluble forms, with maltose as final product. It is one of the most important components of the oral fluid, which exhibits various biological functions, related to its high affinity for binding to oral streptococci and carbohydrate digestion.(1-3) The enzyme has a site for binding to enamel surface and provides also potential sites for the binding of bacterial adhesives. Binding of salivary alpha-amylase to oral microorganisms might be important for the acid production mechanisms of dental plaque, by facilitating starch hydrolysis inside the biofilm and consecutively providing glucose to microorganisms located in the vicinity of tooth structures. In this way, the enzyme could play an important role in enamel demineralization, due to production of acids in the vicinity of tooth structures through the reactions of bacterial metabolism.(4,5) As the physiological functions of alpha-amylase have not been completely explored, there is a growing interest in the use of saliva as an indicator in the prediction, diagnosis and progression of different local and systemic disease, including dental caries.(6-8)

PURPOSE

The purpose of our study is to evaluate the correlation between the salivary level of alpha-amylase and the incidence of dental caries in young permanent teeth.

MATERIALS AND METHODS

A total of 165 school children aged between 9-12 years old were examined at the Center for Integrated Dental Medicine of the Faculty of Dental Medicine, University of Medicine and Pharmacy Tîrgu-Mureş, from October 2016 to June 2017 and consecutively enrolled in this study. Prior to any medical procedure, the parents were asked to give a written consent and the investigation was conducted based on principles

of the Declaration of Helsinki. Children with a history of systemic disease, use of medication during the last 3 months before examination or poor oral hygiene were excluded from the study. Finally, 142 participants were included in four study groups, as follows: group A-caries free girls, Group B-caries active girls, Group C-caries free boys, Group D-caries active boys. The clinical examination was based on visual detection of dental caries based on the following scores: 0- absence of caries, 1-change in enamel aspect, 2- enamel breakdown but no dentin alteration, 3- presence of underlying dark dentin, 4- presence of a distinct cavity, 5- presence of a large cavity. The scores from 0-2 were noted as absence of caries and scores from 3-5 as dental caries present; only when at least 3 teeth had these scores the participant was included in the caries active group. A total of 10 ml of whole un-stimulated saliva was collected in an average 10 min interval, during the morning. The participants were asked to brush the teeth and restrain from drinking or eating for at least 90 min prior to sample collection. Saliva was kept at 4°C and within 20-30 minutes was transferred to the laboratory for the measurement of salivary alpha-amylase levels. The samples were centrifuged for 3-5 minutes in order to obtain pure saliva and enzyme activity was measured using a spectrophotometer (6300, Jenway, Staffordshire, UK) at a wavelength of 590 nm. The reaction of alpha-amylase on a chromogenic substrate produced a colored solution of p-nitrophenol, which darkness is proportional with the level of enzyme activity. The results were expressed in Units per milliliter (U/ml). The collected data were statistically analyzed with the GraphPad Prism 7.03 and the Mann-Whitney test was used to determine the associations between clinical and laboratory observations. A value of $p < 0.05$ was considered to be statistically significant.

RESULTS

The clinical examination revealed a higher number of boys (77cases, 54.3%) with scores between 3-5 for presence of dental caries compared to girls (65 cases, 45.7%) which was

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CLINICAL ASPECTS

significantly different ($p < 0.05$). There were no recordings of caries absence, corresponding to the score of 0 in the caries free groups. The levels of salivary alpha-amylase recorded for each of the four study group are presented in table no. 1 and table no. 2. In caries active groups, the levels of salivary alpha-amylase are significantly higher compared to the groups of caries free children, with the mean values and standard deviations being more different in the boys' groups. There were no significant differences between the salivary levels of the enzyme in caries free and caries active groups according to gender ($p > 0.05$), but children with caries showed a higher amount of alpha-amylase in the un-stimulated whole saliva. The statistical evaluation using Mann-Whitney test showed the distribution of mean and standard deviation for the levels recorded in the study groups, with higher levels for group B and D. The lowest value was recorded in group C and the highest in group D (figure no. 1). Salivary alpha-amylase was significantly higher in the caries active groups ($p < 0.05$, $p = 0.001$) but the mean levels were not significantly different according to gender for caries free and caries active children with p values of 0.63 and 0.26 ($p > 0.05$) respectively.

Table no. 1. The levels of alpha-amylase in the study groups as mean and standard deviation

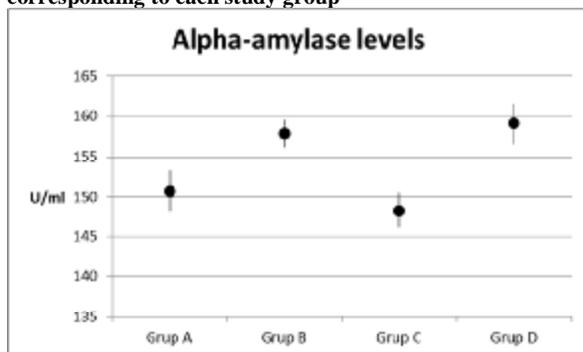
Group	Number	Description	Alpha-amylase level
A	24	Caries free girls	150.53±2.45U/ml
B	41	Caries active girls	156.83±1.59U/ml
C	21	Caries free boys	147.28±2.1U/ml
D	56	Caries active boys	158.18±2.41U/ml

Table no. 2. Comparison between the salivary levels of alpha-amylase among study groups

Variable	Caries free	Caries active	p-value
Boys	147.28±2.1U/ml	158.18±2.41U/ml	0.001*
Girls	150.53±2.45U/ml	156.83±1.59U/ml	0.001*
p-value	0.63	0.26	

*statistically significant difference ($p < 0.05$)

Figure no. 1. Mann-Whitney test with the mean values and standard deviation for the salivary alpha amylase levels corresponding to each study group



DISCUSSIONS

Dental caries is a disease with increasing severity and tooth destruction which has a wide range of clinical aspects, from microscopic alterations of dental hard tissues to the presence of deep lesions.(9) Until recently, little attention has been given to the balance between demineralization and remineralization of dental enamel and the biochemical characteristics of microorganisms present inside the oral biofilm, attached to dental hard tissues. A better understanding of mechanisms of acid production by oral microorganisms based

on the evaluation of saliva, already considered an important noninvasive test for many local and systemic disorders, might reduce cases with advanced disease where the only option of preserving the tooth is endodontic therapy.(9,10) The rate of un-stimulated salivary flow is the most important parameter: parotid glands contribute with 25%, submandibular with 60% and sublingual with 8%. Some studies pointed out that a reduced salivary flow must be considered an important factor that favors dental caries development.(11) The results of our study showed that the mean values of salivary alpha-amylase levels were not statistically different according to gender but the recordings were higher in all caries active groups of children. This observation emphasizes the positive correlation between the salivary level of the enzyme and the presence of carious lesions. The saliva produced by parotid glands represents 25% of the whole saliva and is rich in alpha-amylase. The submandibular glands produce a viscous and rich in mucine secretion and the sublingual glands are pure mucosal. The three-dimensional atomic structure of salivary alpha-amylase has been determined in order to understand the relationship with the functions of this enzyme, the most important being the role in hydrolysis of alpha-1,4-glycosidic linkage in starch and other related polysaccharides.(12) Furthermore, another important property is the high affinity to bind both to oral streptococci and dental hard tissue, which represents the fundamentals for the implication of the high levels of this enzyme as a risk factor for the development of dental caries. Oral bacteria coated with alpha-amylase produce acid by fermentation of starch, but *Streptococcus mutans* alone could not use starch directly unless other microorganisms coated with this enzyme release glucose, which is further used for acid formation.(13) When this process is developing inside the dental biofilm, there is a direct contact of acid with dental hard tissue, leading to enamel demineralization and development of dental caries.(12) The correlation between development of dental caries and salivary levels of alpha-amylase was a matter of intense debate in the scientific literature and the results of different studies are controversial. Results similar to ours were mentioned by other investigators, some of them comparing also the salivary and serum levels of alpha-amylase in group of children with different caries activity.(13) On the contrary, some authors found no strong correlation between the salivary levels of alpha-amylase and early childhood caries and furthermore, an inverse effect was mentioned.(14) Singh et al (15) considered that the protective functions of ptyalin are directed towards keeping the tooth integrity, by cleaning, retarding demineralization, rapidly neutralization of acids produced by oral microorganisms and promoting remineralization. Therefore, we consider that future clinical studies should address this matter and based on the evaluation of large study groups and more clinical and biochemical parameters to offer a better evaluation of the role of salivary alpha-amylase in the initiation and development of dental caries.

CONCLUSIONS

The evaluation of salivary alpha-amylase level could be a helpful method for the determination of individuals with high risk of dental caries as the level of this enzyme increases in caries active patients; furthermore, saliva can be considered an important diagnostic fluid, offering a noninvasive technique for the evaluation of numerous biomarkers, offering new perspectives for preventive dentistry. Systematic methods of caries detection and further risk management studies might contribute to the reduction of caries incidence or the arrest of the disease process.

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