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The relationship between the level of salivary alpha amylase activity and pain severity in patients with symptomatic irreversible pulpitis

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Objectives: Assessment of dental pain severity is very challenging in dentistry. Previous studies have suggested that elevated salivary alpha amylase may contribute to increased physical stresses. There is a close association between salivary alpha amylase and plasma norepinephrine under stressful physical conditions. The aim of this study was to evaluate the relationship between pain severity and salivary alpha amylase levels in patients with symptomatic irreversible pulpitis. ***Materials and Methods:*** Thirty-six patients (20 females and 16 males) with severe tooth pain due to symptomatic irreversible pulpitis were selected. The visual analogue scale (VAS) score was used to assess the pain severity in each patient. Unstimulated whole saliva was collected, and the level of alpha amylase activity was assessed by the spectrophotometric method. Statistical analysis was performed using SPSS 13. ***Results:*** The level of alpha amylase was significantly increased in the saliva in association with pain severity assessed by VAS. The salivary alpha amylase was also elevated with increased age and in males. ***Conclusions:*** There was a significant correlation between the VAS pain scale and salivary alpha amylase level, which indicates this biomarker may be a good index for the objective assessment of pain intensity. (*Restor Dent Endod* 2013;38(3):141-145)

Key words: Alpha Amylase; Pain; Pulpitis; Saliva

Introduction

Pain is an unpleasant feeling conducted via the sensory neurons to the brain.[1] Although pain mostly occurs due to injuries, it can also be initiated by psychosocial causes or referred from other sites. Therefore, difficulty sometimes exists in finding its original cause.[2] Pulp exposure, which may happen after dental caries or acute trauma, leads to pulp damage, which may be followed by intermittent or spontaneous pain. Significant temperature changes could induce long and severe pulpal pain, which may even be prolonged after the removal of the stimulant. The pain will be more severe and localized if inflammation spreads to the periodontal ligament and the surrounding bone.[3]

Saliva has many proteins, and 50 - 60% of salivary protein consists of alpha amylase.[4] Amylase, an important salivary protein, has been suggested as a potential biomarker of the sympathetic reaction to psychosocial stress and parasympathetic responses.[5-7] Although salivary amylase is related to the level of catecholamine in plasma, it cannot be considered a comprehensive indicator of the catecholamine level.[5-7] Shirasaki *et al.* found a significant relationship between pain and the level of salivary alpha amylase.[8]

Regarding the subjective origin of pain, it is difficult to report its character or severity and to identify all of it sources. In the situation in which no physical cause can be found for pain or when the pain severity reported by the patient is not in accordance with visible injuries, the assessment of salivary alpha amylase may help the clinician to recognize the severity of the pain.[9] Additionally, a non-invasive salivary sample collection in a stress-free atmosphere and the ability to collect numerous samples without limitation makes saliva a good measure for the evaluation of catecholamines.[10] To our knowledge, no study has previously been performed to evaluate the relationship of alpha amylase, particularly salivary alpha amylase, to pain due to symptomatic irreversible pulpitis.

Given the limited number of studies performed to evaluate the relationship of alpha amylase, in particular salivary alpha amylase, with pain from symptomatic irreversible pulpitis, the aim of this study was to evaluate the relationship between salivary alpha amylase and pain severity in patients with symptomatic irreversible pulpitis.

Materials and Methods

Study design

This study was a descriptive cross-sectional investigation. Informed consent was obtained from each individual before taking samples. All protocols and patient informed consent forms were approved by the Ethics Committee of Hamadan University of Medical Sciences (Approval number: p/16/35/349). In the present study 36 patients (including 20 females and 16 males) who presented to the dental health centers of Hamadan with severe pain due to symptomatic irreversible pulpitis were recruited to participate in this study. Patients with severe acute tooth pain were included in this study, but participants who smoked, had any systemic diseases, or were on any medication were excluded. Before taking a saliva sample, informed consent was obtained from each individual who sought dental care in the dental health centers of Hamadan and decided to participate in the study.

Clinical examination

Dental examination was performed by a trained dentist. Symptomatic irreversible pulpitis was diagnosed through its symptoms, which included spontaneous sharp and severe pain that was prolonged after removal of the stimulant. Dental radiography was also used as a part of the special investigations. Prior to anesthesia, pain severity was assessed using a visual analogue scale (VAS) and 5 mL of unstimulated saliva was taken from each patient in a sitting position while bending the neck forward via the spitting method.[3,11,12]

Sialochemical analysis

The salivary samples were centrifuged (KD2-TDSA, Nantong Hailun Bio-medical Apparatus Manufacturing C., Ltd., Haimen city, Jiangsu, China) for 3 - 5 minutes in order to acquire pure saliva. The level of alpha amylase activity in the saliva was assessed 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 colored solution of chloro-p-nitrophenol, and its darkness was proportional to the level of enzyme activity.[13,14]

Statistical analysis

Data were analyzed using SPSS version 13. The normal distribution of the data was evaluated by the Kolmogorov-Smirnov test. The t test was used in order to compare the level of alpha amylase activity according to sex. For the assessment of the relationship of alpha amylase activity with age and pain severity, Spearman correlation coefficients were used. Multiple regressions were applied to detect the most important factors affecting the level of salivary alpha amylase activity (unit/mL). The covariates of this study were pain (VAS score), gender (0, female; 1, male), and age (years). The regression model was fitted with the backward method, and then the final model with significant covariates was reported.

Results

In this study, 55.6% of patients were female and 44.4% were male. Descriptive analyses of age and alpha amylase activity are shown in Table 1. The difference in the salivary alpha amylase activity between the males and females was not significant (Mann-Whitney U-test, $p = 0.116$), and there was no significant difference in the pain severities between the males and females (Mann-Whitney U-test, $p = 0.838$). According to the VAS scores, 50% of the patients had a pain severity higher than 6. The distribution of pain severities was as follows: 3 (2.8%), 4 (5.6%), 5 (13.9%), 6 (27.8%), 7 (11.1%), 8 (8.3%), 9 (13.9%), and 10 (16.7%). The Spearman correlation analysis showed statistically significant correlations between age, pain severity, and alpha amylase activity. Pain showed a positive correlation with age ($r = 0.447$, $p = 0.006$, Table 2). The salivary alpha amylase level was directly correlated with age ($r = 0.553$, $p = 0.001$) and pain severity ($r = 0.358$, $p = 0.032$, Table 2).

Multiple regression analyses also demonstrated that the level of salivary alpha amylase activity increased along with age. Older patients had more severe pain than younger patients. The increase of a year in age raised the salivary alpha amylase by 2.898 unit/mL (Table 3). After

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Table 1. The mean and standard deviation (SD) of the age and the level of salivary alpha-amylase activity of the participants

	Number	Age	Salivary alpha amylase activity (unit/mL)
Female	20	35.1 ± 24.6	105.28 ± 62.70
Male	16	33.1 ± 11.7	141.65 ± 46.54
Total	36	34.2 ± 11.4	122.21 ± 58.29
p value*		0.116	0.838

*Mann-Whitney U test.

Table 2. The Spearman correlation coefficient between age, pain, and alpha amylase activity

	Age	Salivary alpha amylase activity
Pain	$r = 0.447$*	$r = 0.358$
	$p = 0.006$	$p = 0.032$
Salivary alpha amylase activity	$r = 0.553$	
	$p = 0.001$	

*Spearman correlation coefficient.

Table 3. The results of the multiple regression model

Variable	Beta	Standard deviation	Wald statistic	*p* value
Age	2.89	0.68	4.22	< 0.001
Gender	42.13	15.47	2.72	0.010

adjusting for age, gender was also a factor affecting the alpha amylase activity level and the males had higher alpha amylase levels than females. The salivary alpha amylase activity of men was 42.139 times greater than that of women (Table 3). Table 3 shows that the level of alpha amylase activity was related more to the age and sex than the pain severity.

Discussion

In the present study, the level of salivary alpha amylase activity of patients with mild to severe pain was found to have a wide range of 6.30 to 241.50 unit/mL. Similarly to previous studies,[8,15] the results of this study showed that the level of salivary alpha amylase was proportional to the level of pain reported by patients. Alpha amylase activity differed between male and female participants. In contrast to female participants, an increase in the level of salivary alpha amylase activity up to 42.131 unit/mL was observed in male participants. In addition, the level of salivary alpha amylase activity increased along with age, that is, an increase of one year in age was associated with an increase in salivary alpha amylase by 2.898 unit/mL.

Because previous studies have demonstrated that some diseases, including diabetes, bulimia, anorexia, renal diseases, chronic pancreatitis, and celiac disease, increase the level of alpha amylase activity, those who had systemic diseases were excluded from the present study.[16-18] As smoking and alcohol consumption affect the salivary flow rate and its composition, people with such habits were also excluded from the study.[19,20]

Similar to the results of the current study, Shirasakish *et al.* and Noto *et al.* showed that there were correlations between the pain severity assessed by VAS and the salivary alpha amylase level.[8,21] The studies also concluded that the level of salivary alpha amylase could be a reasonable biomarker for objective evaluation of pain. Bugdayci *et al.* showed that in patients with migraine pain the level of salivary alpha amylase was elevated. Limited studies have been performed to evaluate the level of salivary alpha amylase in men and women.[15] However, Enberg *et al.* and Dezan *et al.*, in contrast to the results of the present study, found no difference between the salivary alpha amylase activity by gender.[22,23] Enberg *et al.* concluded that salivary alpha amylase activity was sensitive to psychosocial stimulation. They also showed that the relationships of both genders to the stimulation were the same.[22]

The results of previous studies revealed that the coordination of the sympathetic branch of the autonomic nervous system and the secretion of alpha amylase in the saliva increased from 2 to 6 months of age.[24,25] The level of salivary alpha amylase activity of 6- to 12-month-old children was relatively equivalent to the adult level.[26] In another study, Inukai found that there was a positive relationship between age and salivary alpha amylase activity, which confirms the results of the present study.[27]

Another study on salivary alpha amylase activity and pain-induced stress in orthodontic patients showed that there

was no correlation between alpha amylase concentrations in the saliva and pain intensity, although the patients had a significant and progressive increase of alpha-amylase levels daily, before treatment, after bracket bonding, and after initial arch wire insertion.[28] The limitations of the current study include absence of similar studies and no use of a control group for comparison. Future studies need to include salivary samples from individuals with a comparable extent of dental caries, age, nutritional habits, oral hygiene status, and psychosocial situation.

Conclusions

According to the results of the present study, the level of salivary alpha-amylase was significantly correlated with the pain severity assed by VAS. Therefore, based on the ease of its evaluation, it could be considered a biomarker for pain. However, future studies are strongly recommended for the assessment of probable factors that may affect its activity.

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