Original Article

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The Effects of Dominant Chewing Side on Amylase Enzyme Activities in the Parotid Gland

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ABSTRACT

BACKGROUND AND OBJECTIVE: Salivary secretion is almost entirely regulated by the nervous system. This secretion is mostly increased in response to gustatory sensations and jaw movements during chewing. This study was conducted to evaluate the effects of dominant chewing side on amylase enzyme activities.

METHODS: Overall, 48 healthy individuals within the age ranges of 6-12 and 41-61 years, with no history of cell phone use, took part in this experimental study. The saliva samples of both chewing sides were obtained separately from the Stensen's foramen, using a capillary tube. The level of amylase activity (U/L) was evaluated, based on the dominant chewing side.

FINDINGS: As the results indicated, 28 subjects preferred the right side for chewing and 20 subjects frequently chewed on the left side. The mean amylase activity in the former group was 27901±38141 U/L on the right side and 60954±51325 U/L on the left side (p=0.000). The mean amylase activity in the latter group was 52995±39857 U/L on the right side and 25464±22344 U/L on the left side (p=0.000). There was a significant relationship between amylase activity and the dominant chewing side (p<0.001).

CONCLUSION: The results of this study showed that the activity of amylase enzyme, secreted from the parotid glands, decreases in the dominant chewing side.

KEY WORDS: Amylase, Chewing, Parotid Glands.

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Introduction

Alpha amylase, as a digestive enzyme, is secreted from the parotid glands, submandibular glands and exocrine pancreas and is found in the saliva of humans and other mammals. This enzyme starts the digestive process in the mouth and plays a role in converting polysaccharides (e.g., starch and glycogen) into disaccharide (maltose) and dextrin.

Amylase is the most common protein secreted from the parotid glands. Almost all secretions in parotid glands are exclusively of serous type. The amount of amylase in saliva depends on genetic and environmental factors and varies in different individuals (1). Through stimulating the autonomic nervous system, salivary alpha amylase is locally secreted by acinar cells in the exocrine parotid glands and submandibular glands (mostly secreted by the parotid glands) (2). Salivary secretion is almost entirely regulated by the nervous system. This secretion is mostly increased in response to gustatory sensations and jaw movements during chewing (3). The stimulation of sympathetic nerves leads to the secretion of low-volume, enzyme-loaded saliva, which is thick and viscous in nature (4).

Alpha amylase shows an apparent daily decrease within 60 minutes after waking up and a constant increase in activity during the day (5). The salivary gland consists of secretory end pieces (acini) and the conduit system, which varies in size depending on the gland. During the stimulation of secretory end pieces, initial isotonic saliva is released with an ionic composition (sodium chloride), similar to plasma. This substance goes through some changes while passing through the conduit system by selective reabsorption of chloride and sodium (water-free) and secretion of potassium and bicarbonate. The final salivary compound, which is secreted into the oral cavity, is highly dependent on salivary flow rate.

When the salivary flow rate increases, the concentration of total protein, sodium, total calcium, chloride and bicarbonate increases, whereas the concentration of total phosphate decreases. Overall, the salivary flow rate and subsequently its compounds are influenced by the type and size of salivary glands, hydration state, nutritional status, sample collection time, nature and duration of stimulation, emotional state and gender (6).

The saliva secreted from the parotid glands has a higher protein content in response to salty stimuli, compared to acidic stimuli (7). There are various reports on the relationship between aging and salivary flow rate. However, it is generally believed that aging in healthy individuals would not decrease the salivary flow (6). Particularly, the whole salivary flow rate and parotid stimulatory and non-stimulatory flow rates remain constant with aging (6).

In addition to its gastric function, alpha amylase plays an important antibacterial role (8). The production of alpha amylase as a biomarker of the activity of autonomic nervous system has been applied in different areas of biological research (9). Several studies have been conducted on the use of salivary amylase as a criterion for the activity of autonomic nervous system, objective pain assessment and treatment of type II diabetes (10, 11).

This enzyme has been also used in previous studies as a biomarker of psychological stress (9). Alpha amylase connects to Streptococcus and contributes to the regulation of bacterial binding to oral surfaces; this is why alpha amylase is considered to play a role in oral health (6). One parotid gland lies in front of each ear and secretes its content through Stensen's foramen into the oral cavity in the vicinity of maxillary first molar.

Physical and psychological stress can affect the secretion of amylase enzyme. Chewing is an important physiological factor for salivary secretion (12). Gopal et al. stated that increased salivary amylase activity in response to emotional stress is higher in younger people. Also, men have a higher level of salivary amylase, compared to women (13). In this regard, Padmavati et al. concluded that amylase activity decreases with aging and amylase synthesis and secretion are higher in men than women (14). In a study by Hector et al., which evaluated the effects of chewing side preference on the secretion of parotid amylase in rabbits, no significant association was observed between amylase concentration and chewing side preference. However, they suggested that the total amylase output was significantly higher on the preferred chewing side, compared to the opposite side (15). Mackie et al. concluded that chewing increases the salivary flow rate, not the concentration of proteins or alpha amylase (16). Moreover, Losso et al. showed that those who mostly chewed on their left side had a significantly higher salivary flow rate on that side, compared to the control side (opposite side).

In this regard, Arhakis et al. concluded that the amount of salivary amylase in the stimulatory state (chewing unsweetened gum) was on average 1.5 times

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higher than the resting state, while the salivary flow rate increased by 6.3 times. It should be mentioned that no individual chews equally on both sides, and the pattern varies in different people (6).

The main objective of this study was to compare the dominant chewing side and amylase enzyme activity. In case our findings indicate the influence of dominant chewing side on amylase activity, the dominant chewing side should be considered as an important factor in studies on the effects of environmental factors on amylase activity.

According to the available data, no previous studies have noted the effect of dominant chewing side on amylase activity (17, 19). Therefore, the current study was performed to evaluate the effects of dominant chewing side on the activities of amylase enzyme, secreted from the parotid glands.

Methods

Overall, 48 healthy individuals within the age ranges of 6-12 and 41-61 years, with no history of cell phone use, took part in this experimental study. The saliva samples of both chewing sides were obtained separately from the Stensen's foramen, using a capillary tube. The level of amylase enzyme activity (U/L) was evaluated, based on the dominant chewing side. The exclusion criteria were as follows: 1) use of medicines affecting the salivary glands; 2) use of medications leading to a decrease or increase in the salivary flow rate (e.g., antihypertensive and antidepressant drugs); 3) use of medicines affecting the gastric system or causing xerostomia; 4) frequent cigarette smoking (20 or more cigars per day) or alcohol use (20); 5) diseases affecting the salivary glands such as connective tissue diseases (Sjögren's syndrome, rheumatoid arthritis and chronic systematic diseases); 6) a prior history of trauma to the head and neck; 7) pregnancy or anemia; 8) not having a dominant chewing side (17); 9) use of any orthodontic appliances such as braces and full/partial denture; 10) periodontitis (18); and 11) any oral complaints or clinical signs.

A checklist was prepared containing the following information: the subject's gender, age, general health (absence of chronic diseases such as diabetes or autoimmune diseases), no chronic use of drugs, cigarettes or alcohol, and the dominant chewing side. The subjects were asked to visit the hospital two hours after breakfast, between 9 a.m. and 12 a.m. to collect the saliva samples. They were asked not to drink, eat or smoke cigarettes one hour before sampling. The subjects were asked to wash their mouths with water and lay down on the dental unit in a calm state, under adequate lighting.

The Stensen's foramen was dried with sterile gauze. A thin glass capillary tube (a laboratory microhematocrit tube), which is open on both ends and can suck up almost one-tenth of a milliliter of saliva due to capillarity, was placed in the vicinity of the conduit. Saliva was secreted into the tube by gentle squeezing of the parotid gland. After reaching the desired volume (almost half of the tube), the other end of the tube was sealed with a special paste to prevent the excretion of saliva. This procedure was performed on the opposite side of the salivary gland, as well. Each patient was given a number from 1 to 48, and the saliva-containing tubes were marked according to the patient number and left/right chewing side. The tubes were kept in the refrigerator before being sent to the lab. At this point, a gum was given to each subject for chewing. The findings related to the dominant chewing side were recorded on the checklist in order to keep an accurate record of the dominant chewing side.

The activity level of amylase (U/L) was assessed in the laboratory, using Pars Azmoun Amylase Kit (Pars Azmoun, Tehran, Iran), with the help of an autoanalyzer. The findings were analyzed, using t-test, paired test, Mann-Whitney and Wilcoxon test. P-value less than 0.05 was considered statistically significant.

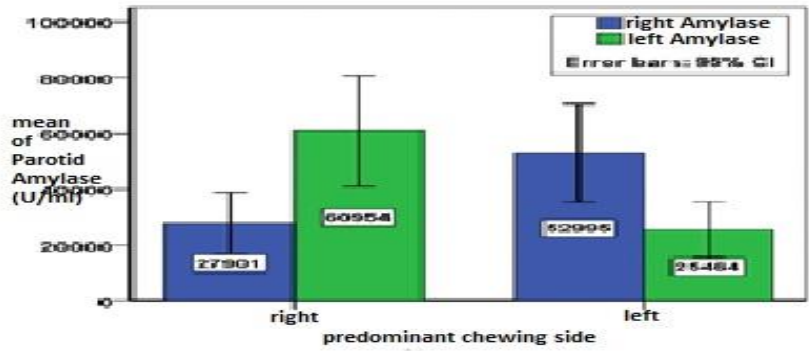
Result

In this study, 28 subjects mostly chewed on their right side and 20 subjects frequently chewed on their left side. The mean amylase activity in the former group was 27901±38141 U/L on the right side and 60954±51325 U/L on the left side (P=0.000). The mean amylase activity in the latter group was 52995±39857 U/L on the right side and 25464±22344 U/L on the left side (p=0.000) (fig 1).

There was a significant relationship between amylase activity and the dominant chewing side ($p<0.001$). In fact, amylase activity in the dominant chewing side was lower than the opposite side. Non-parametric tests also confirmed these findings. Also, no significant relationship was found between amylase activity and gender.

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Figure-Table 1. The comparison of dominant chewing side and mean amylase activity in the parotid glands (U/L)



Variables	Amylase secreted in the right side (Means±SD)	Amylase secreted in the left side (Means±SD)	P-value**
Right side dominance	27901±28141	60954±51325	0.000
Left side dominance	52995±39857	25464±22344	0.001
P-value*	0.012	0.002	

*t-test, **paired t-test

Discussion

The results of this study showed that amylase enzyme activity in the dominant chewing side is lower than the opposite side. There was no relationship between amylase concentration and gender. However, unlike the present study, Gopal et al. found that increased salivary amylase activity in response to psychological stress was higher in younger people. Also, men had a higher level of salivary amylase, compared to women (13).

Padmavati et al. concluded that amylase activity decreased with aging. Also, amylase secretion and synthesis were higher in men, compared to women (14). It should be mentioned that total saliva was assessed in the mentioned studies, while in the current research, we evaluated saliva secreting from the parotid glands. As previously mentioned, the amount of salivary compounds is influenced by the type and size of salivary glands, hydration state, nutritional state, time of sample collection, nature and duration of stimulation, emotional state and gender (6). Since it is not possible to match all these features in all study groups, this type of conclusion cannot be without error. Also, the discrepancy in the results of mentioned studies and our research can be due to differences in sample collection and storing methods. It is still unclear if the parotid gland can constantly secrete enough amylase or whether the synthesis of stimulated amylase is fast enough to replace the secreted amount (21). However, the parotid gland has been shown to keep the protein output high for a long period (21). Several factors such as stress, depression, age, alcohol use, exercise, and radiotherapy might affect the salivary flow rate. Fluctuations in the concentration of salivary amylase coincide with changes in the salivary flow in all mentioned studies. Nonetheless, it is still unclear if the fluctuations of amylase concentration are due to some changes in the salivary flow rate or there are other mechanisms affecting the salivary flow rate and salivary compounds during chewing. The most possible mechanism seems to be the stimulation of parotid flow by chewing through mechanical receivers, which are located in the periodontal ligament, gum tissues and the tongue (22). The mechanism of protein secretion, which is controlled by the nervous system, is not fully determined. However, it seems that the sympathetic system plays the main regulatory role in low salivary flow rate. Also, granule exocytosis is considered a more active supply; in fact, both systems contribute to salivary reflexes. Parasympathetic system leads to an increase in the flow rate. Also, it has stimulatory effects on the secretion of non-storage granules. This system consequently leads to a decrease in the secretion and synthesis of protein, but the high amount of water output neutralizes the increase in protein synthesis (22). Another mechanism that can increase the salivary flow rate in the dominant chewing side is probably the growing and increased gland activities in the dominant chewing side (7). The hypothesis of reduction in amylase activity due to the increase in salivary flow is more plausible. In support of this hypothesis, Hector et al. by evaluating the

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effects of chewing side on the secretion of parotid amylase in rabbits showed no difference in amylase concentration between the dominant chewing side and the opposite side. However, they indicated that the total amount of amylase output was significantly higher on the chewing side, compared to the other side (15). Although these researchers indicated no significant difference in amylase concentration between the two sides, they suggested that amylase output was higher on the chewing side, which is due to an increase in the salivary flow rate. In other words, the chewing side has a higher flow rate than the opposite side. This conclusion seems logical as salivary secretion increases in response to gustatory sensations and chewing (3), and jaw movements are more in the chewing side. Mackie et al. concluded that chewing increases the salivary flow rate, not protein or alpha amylase concentrations (16). They suggested that chewing itself induces the secretion of saliva, proteins, and alpha amylase. Also, chewing food secretes a higher volume of saliva than chewing paraffin. The amount of secretion was significantly increased with doubling the food weight. Also, the amount of secretion was different for equal weights of different foods (16). Mackie et al. also concluded that chewing has no effects on salivary response (16). The considerable effect of chewing on the pattern and level of salivary response suggests that other senses can stimulate the parotid gland alone or along with the sense of taste (23).

This study also confirmed that chewing increases salivary secretion; in this study, total saliva was used for sampling. Jensen et al. after evaluating fluid, amylase and kallikrein discharge during reflux stimulation in the normal state (without any drugs) and after acute administration of autonomic blocking agents showed that chewing increased salivary products. Also, when the duration of chewing extended, the fluid discharge increased, not the enzyme secretion; in fact, taste stimuli significantly increased amylase and kallikrein discharge. It was concluded that saliva-chewing reflex mostly activates the parasympathetic pathway, which leads to the production of low-protein saliva (24). Losso et al. evaluated the effects of acidic taste stimuli on the flow rate and protein content of saliva in the dominant chewing side. They concluded that those who chewed dominantly on the left side had a significantly higher salivary flow rate on that side, compared to the control side (the opposite side). These findings were in accordance with our study findings. However, Losso et al. suggested that the mean protein concentration on the left side in those who preferred to chew on the right side was higher, which was not statistically significant (7). This finding did not apply to people who chewed on their right side. A reverse relationship was indicated between salivary flow rate and protein concentration. Arhakis et al. in 2013 indicated that the amount of salivary amylase in the stimulatory state (chewing unsweetened gum) was on average 1.5 times higher than the resting state, whereas the salivary flow rate increased by 6.3 times. They concluded that salivary flow rate is a mediatory factor in measuring salivary amylase. Gallia et al. in 2011 asserted that changes in salivary secretion constitute for 25-40% of changes in salivary amylase (26).

Also, Rohleder et al. in 2006 showed that the increase in amylase concentration due to social and emotional stressors elevated salivary alpha amylase, regardless of the salivary flow rate (25). They concluded that in the absence of distressing stimuli, the salivary flow rate, age and the interaction between these two factors can affect amylase secretion in young adults (25). In 2009, Neyraud et al. showed that with mechanical stimulation, parotid salivary flow rate and pH increased linearly, while protein concentration linearly decreased with an algorithmic increase in the salivary flow rate (22). All these studies indicated that chewing increases the salivary flow rate (15, 16, 21, 22, 24-26). As mentioned in the study by Arhakis, salivary flow rate is a mediatory factor in measuring salivary amylase (26). It is unclear to what extent the increase in salivary flow rate and decrease in amylase secretion contribute to this conclusion. However, the role of increased salivary flow rate in the dilution of amylase and consequently the reduction of its activities on the chewing side is considerable; it seems that this increased rate is more important than the reduction in amylase secretion. According to the results of this study, dominant chewing on one side led to a reduction in amylase activity on the same side. This finding showed that chewing is an important mediatory factor. Salivary flow rate was higher in the dominant chewing side due to the higher function of the glands. Also, it seems that the salivary flow rate led to the higher dilution of amylase enzyme.

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