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Original Article

Evaluation of the effect of handheld mobile phone use on activity of the parotid glands amylase enzyme

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Abstract

Introduction: Alpha amylase is the most abundant enzyme of parotid gland. This enzyme starts digestion of carbohydrates in the mouth. A pair of parotid glands is located in front of ears. Several studies have been conducted on the effects of mobile phones on the parotid gland. The aim of this study was to investigate the effects of mobile phones on the activity of the amylase enzyme.

Materials &Methods: Totally, 251 men and women aged 18 to 55 years, who only used Hamrahe Aval's operator were included. Parotid salivary samples of each individual were bilaterally collected using capillary tube placed in the vicinity of Stensen's duct. Bilateral amylase activity of each individual was evaluated with spectrophotometric method in the laboratory considering dominant sides of phone conversation and chewing. Data were analyzed using Two-way ANOVA, $\chi 2$, T test and SPSS software.

Results: The relationship between the dominant phone conversation side and parotid amylase activity was not statistically significant but the correlation between prevailing chewing side and amylase activity was statistically significant (p=0.001).

Conclusion: Handheld mobile phone was not effective on parotid amylase enzyme activity whereas chewing was effective on parotid amylase enzyme activity.

Keywords: Chewing, Mobile phone, Parotid gland, Amylase

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بررسی اثر استفاده از تلفن همراه بر فعالیت آنزیم آمیلاز غدد پاروتید

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چکیده

مقدمه: آلفا آمیلاز فراوانترین آنزیم غدد پاروتید است. این آنزیم هضم کربوهیدراتها را در دهان آغاز می کند. تحقیقات متعددی در مورد اثر تلفن همراه بر غده پاروتید انجام شده است. هدف این تحقیق بررسی تاثیر تلفن همراه بر فعالیت آنزیم آمیلاز غده پاروتید بود.

مواد و روش ها: ۲۵۱نفر مرد و زن در گروه سنی۱۸ تا ۵۵سال که از لحاظ سیستمیک سالم بودند و فقط از اپراتور همراه اول استفاده می نمودند، وارد طرح شدند نمونه های بزاق پاروتید دو طرف هر فرد توسط لوله مویینه ای که در مجاورت مجرای استنسن قرار می گرفت، جمع آوری شد. میزان فعالیت آمیلاز دو طرف افراد با در نظر گیری سمت غالب مکالمه و سمت غالب جویدن در آزمایشگاه با روش اسپکترو فتومتری سنجش گردیده و داده ها توسط آزمون Two-way ANOVA, $\chi 2$,T test و نرم افزار *SPSS* آنالیز گردید.

یافته ها: ارتباط بین سمت غالب مکالمه و فعالیت آمیلاز پاروتید از لحاظ آماری معنی دار نبوده ولی ارتباط سمت غالب جویدن بر روی فعالیت آمیلاز از لحاظ آماری معنی دار بود($p=0.001$).

نتیجه گیری: تلفن همراه بر فعالیت آنزیم آمیلاز پاروتید موثر نیست در حالیکه جویدن بر فعالیت آنزیم آمیلاز پاروتید موثر است.

واژگان کلیدی: جویدن، تلفن همراه، غده پاروتید، آمیلاز

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Introduction

World Health Organization (WHO) defines health as a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity.[1] The human body responds to many stimuli as a part of life. If these changes are beyond the scope of compensation mechanism, biological effect can lead to health risks (Psychological, physical and social health).[2] Radio frequency (RF) as part of the EM spectrum has a frequency between 100 kHz to 300 GHz.[3]Cell phone waves (a part of radio waves with a range of 800 to 2200 MHz) by its long-term exposure and proximity to the human body cause concerns about possibility of its adverse effects due to absorption by the tissues adjacent to areas where mobile phone is placed.[4] On 31 May 2011 the International Agency for Research on Cancer (IARC) affiliated to World Health Organization (WHO) categorized radio waves in the group 2B (possibly carcinogenic to humans).[5] Non-ionizing radiation International Committee (ICIRC) is responsible for investigating on the risks associated with different forms of non-ionizing radiation. The committee guidelines limit exposure to electric, magnetic and electromagnetic fields. Simultaneously, with the expanding use of communication technology in daily life, discussion about the biological effects of EM waves is on progress. In particular, increasing the use of mobile phones has created lots of concerns about the potential hazards of radio waves on human.[6] Since the mobile phone is in a position very close to body, this concern increases .[3] Parotid glands are located in front of external ear canals and due to the close proximity to the location of mobile phones and lack of physical protection by bones they are highly influenced by mobile phones and these effects are more on the side dominantly used during phone conversation.[4] Parotid gland secretion is mainly serous. The parotid serous protein is called as amylase. The parotid and exocrine pancreas both release amylase.[7] Almost, all of the parotid secretion is of serous type while submandibular and sublingual glands have serous and mucous secretions. Minor salivary glands have only mucus secretions. Normal pH of saliva is 6 - 7 and it is optimal for performance of amylase. Following secretion of saliva by parotid glands it is released in the mouth through Stensen's duct adjacent to the maxillary first molars. Capillaries near the end of salivary secretory

component are very important for the secretion of saliva. They are stimulated by autonomic nervous system. Salivary secretion is almost completely controlled by nervous system. [7]

Alpha-amylase is a digestive enzyme. It breaks high molecular weight polysaccharides such as starch and glycogen and converts them to smaller molecules, glucose and maltose. [8] Salivary alpha-amylase level is not related to blood alpha amylase levels derived from pancreas. [9]

Alpha-amylase production in salivary glands occurs in response to physical and psychological stress under the control of autonomic nervous system.[10] Alpha-amylase levels are used as a bio-marker of autonomic nervous system activity in different areas of biological research.[11] Since amylase converts starch into glucose and maltose, it plays an important role in increasing diet glucose levels. Several studies are performed on the use of salivary amylase as a measure of autonomic nervous system activity, [12-15] objective assessment of pain, [16, 17] and the treatment of type 2 diabetes. [17]

Salivary amylase level is related to inheritance and environmental factors. It varies among individuals.[8] According to the obtained information in studies investigated the effect of mobile phones on the salivary glands, the role of chewing as a confounding factor is not taken into account [18-24] and therefor the results of these studies cannot be attributed to mobile phones. This study is performed to evaluate the effect of mobile phones on amylase activity considering chewing effect. If it is approved that mobile phone is effective on amylase activity, further studies can be performed on the long-term impact of these findings on quality of life.

Materials&Methods

Before the start of main project that aimed at investigating the effects of mobile phones on the parotid gland secretion, a pilot study was conducted to determine the primary relationship between the amylase activity of both sides and the dominant chewing side in individuals with no or limited use of mobile phones. In this pilot study, a total of 50 participants including 35 children and adolescents aged 6-12 years, who did not use cell phones (Group I) and 15 individuals aged 41-61 years (Group II) who had limited use (less than 5 minutes a day) were selected. [21] All subjects were systematically healthy (28 females and 22 males). In the first group, 18 persons used right side, 15 used left side and 2 used both sides to chew. In the second group 10 people mostly chewed from the right and 5 chose left to chew. The parotid salivary samples were collected by the method that will be mentioned in the main plan and amylase activity was measured. Inclusion criteria in this study are that all females and males aged 20 to 60 years who used mobile phones (only Hamrahe Aval operator) with no systemic problem. People who had the exclusion criteria were removed from the plan. Exclusion criteria include: 1) use of any drug or medication that can affect the salivary glands and is capable of reducing or increasing the flow rate of saliva (such as antihypertensive drugs, antidepressants, or drugs that affect the digestive system, or those that cause xerostomia) 2) Heavy smokers (20 or more cigarettes daily) [25]and alcoholics. [3]

3) Conditions affecting salivary glands such as connective tissue disease (Sjogren's disease, RA., Systemic chronic diseases, history of trauma to the head and neck, pregnancy, anemia) 4) Absence of dominant side of phone conversation 5) Use of hands-free and similar tools [24] 6) Use of any dental appliances such as orthodontic wires, complete and partial dentures, etc 7) periodontitis [26] 8)active decay 9) BMI higher than 30 10) Any oral complains with clinical signs and symptoms 11) Use of any operator other than Hamrahe Aval (Hamrahe Aval is the only operator used by individuals included in this study) In the original plan, 251 people had inclusion criteria and were entered into the study. This study was approved by the ethics committee of Babol University of Medical Sciences with code number of 4063.

The written consent form was obtained from individuals and they were invited two hours after breakfast between 9-12 a.m. to collect the saliva samples. One hour before referral participants had to refrain from eating and drinking other than water and did not smoke. They were asked to wash their mouth with water and relax on dental unit with adequate light. Stensen's duct area was wiped with dry sterile gauze. Thin glass capillary tubes (laboratory micro hematocrit tube) which were open on both sides and is capable of pulling up one tenth milliliter of saliva through the capillary rise are placed in the vicinity of the channel (figure 1).

Slow squeeze of parotid gland for several times usually causes the withdrawal of saliva from the glands and its inclusion into the tube. After reaching sufficient volume (half pipe), the other end was blocked with a

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special paste to prevent the outflow of saliva. This process is repeated for the gland of other side. Each person was assigned a number. The tube containing the saliva of each side was attached on the paper marked in terms of patient's number and their left and right side. The tubes containing saliva can be refrigerated until shipment to the laboratory. Age, sex, dominant side used during chewing and phone conversation, duration of daily calls and use of mobile phone were recorded. To increase the accuracy of recording the dominant side, a chewing gum was given to patient without informing them about the goal, they were asked to chew. All observations and oral examinations were recorded.



Figure1. Sampling saliva of parotid by laboratory micro hematocrit tube

In the laboratory, in order to exclude deteriorated cells and other substances that were likely to cause darkening of samples in the auto-analyzer machine they were centrifuged for 5 minutes by micro hematocrit. Transparent samples were diluted 200 times with normal saline.

In the laboratory, amylase activity was measured using Pars amylase test kit (Pars test, Tehran, Iran) by auto-analyzer units (U/ml). Data were divided into three different groups. The first group consisted of people who had an average of 30 minutes or more of mobile phone, phone conversation per day and mostly used their right ear and had mobile phones for at least 7 years.[6] The second group was similar to first group but instead of right ear they mostly use their left ear during phone conversation.

The third group consisted of individuals who did not use mobile phones or used it very less (an average of 10 minutes daily call and from zero to 10 years have a mobile phone). Since the speech and chewing sides may not match, in each group it was tried to approximately maintain the equal or close number of individuals who used the same side for chewing and speech with those who did not use similar side for these purposes in order to statistically examine the effects of the bite on amylase secretion.

Data were analyzed using SPSS software, X2, TTCT, two-way analysis of variance and correlation coefficients and $p<0.05$ was statistically considered significant.

Results

Totally, 251 individuals participated in this study. Out of these, 143 persons (57%) mostly used right side and 108 persons (43%) mostly used left side during phone conversation. Among them, 129 participants were dominant right chewers (4.51%) and 122 of them were dominant left chewers (6.48%). Average amylase activity of right and left parotid glands in individuals dominantly using right or left side to speak and in dominant left and right chewers is shown in table 1.

Table1. Average amylase activity of right and left parotid gland on chewing and phone conversation side

Average of amylase activity(U/L)		N(%)		
Left	right		chewing side	Phone side
138932.86	63843.86	70(27.8)	Right	Right
47736.71	124520.55	73(29.2)	Right	Left
96215.25	57769.15	59(23.5)	Right	Left
42795.92	103832.65	49(19.5)	Left	Left

There was no significant correlation between the phone conversation side and amylase activity on right side ($p=0.410$). While the relationship between chewing side and amylase activity was statistically significant on right side ($p=0.001$), the correlation between phone conversation side and amylase activity was not statistically significant on left side ($p=0.092$). There was a statistically significant correlation between chewing side and amylase activity on left side ($p <0.001$). The relationship between amylase activity and duration of daily mobile use, long years of use, age, and sex was not significant ($p>0.05$).

Figure 2 shows mean difference of amylase activity of right and left side with respect to chewing and phone conversation side. (Similar letters in the figure express absence of significant difference)

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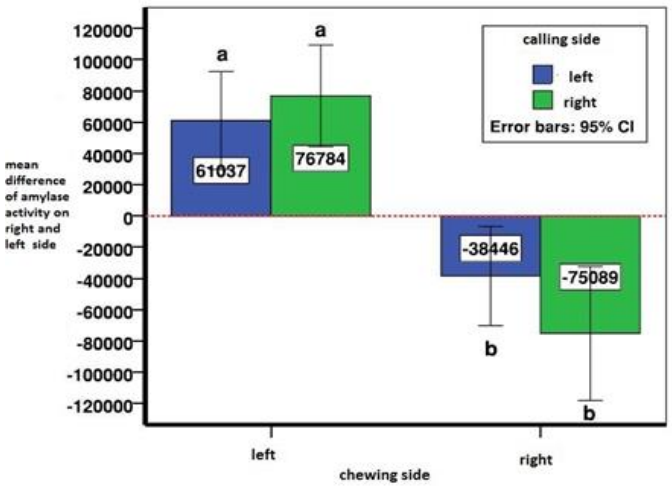

Figure2. Mean difference of amylase activity on right and left side with respect to chewing and phone conversation side

Preliminary results: In the current study, 28 individuals often used right side to chew (right chewer) and 20 of them most often chose left side to chew (left chewer). Mean amylase activity of right chewers on right side was 38141±27901 Unit per Liter and for left chewers was 51325±60954 Unit per Liter ($p<0.001$). Average amylase activity was 39857±52995 Unit per Liter for left chewers on right side and it was 22344±25464 Unit per Liter on left side ($p<0.001$). There was a significant relation between dominant chewing side and amylase activity ($p<0.001$). Results showed that on dominant chewing side, parotid gland amylase activity was decreased.[21]

Discussion

The results of this study showed that unlike the dominant chewing side, the prevailing phone conversation side had no impact on activity of parotid gland amylase. Hashemipour et al. studied on 86 individuals and they concluded that when the right side was the dominant phone conversation side it caused an increase in salivary flow rate and protein concentration level and reduction in amylase, lipase, lysozyme, lactoferrin and peroxidase. In individuals dominantly using left side to speak, salivary flow rate is equal on both sides.[6] At first, these researchers did not consider chewing factor. Secondly, if the mobile phone was effective on amylase activity, why was this impact just observed on those who dominantly used right side during phone conversation? In the present study, reduction in amylase was only observed on dominant chewing side and the phone conversation side was ineffective on amylase activity. (Among those who dominantly used right or left side during phone conversation).

In a similar study performed by Bhargava et al. on 142 healthy subjects aged 18 to 30 years (mean 5.2±21) with at least 3 years of mobile usage (mean 5 years), it was concluded that the use of mobile phones for more than 2 years caused an increase in salivary flow by 26% on the same side and with daily usage of less than 2 hours salivary flow increases by 8 % on the same side.[2] The researchers attributed the increased flow of saliva to the mobile phone while they didn't consider the chewing factor. Despite this important confounding factor, it cannot be entirely attributed to the use of mobile phone. The preliminary investigation in this study showed that amylase activity decreases on dominant chewing side of individuals who do not use mobile phone.

Goldwein et al. on evaluation of 50 healthy volunteers concluded that salivary flow on right side of individuals who dominantly used right side during phone conversation was more than the other side and in dominant left side users equal flow rate was observed on both sides. [1]The result of this study was similar to Hashemipour et al.'s findings. They had same flaws as they did not consider chewing factor and also could not explain cause for impact of mobile phone only on the right side and not on the left side.[6] Physical and psychological stress can affect the secretion of amylase. Chewing is an important physiological stress in secretion of saliva.

Mackie et al in their study on chewing and its impact on salivary flow and alpha amylase secretion on 10 individuals (7 females and 3 males) concluded that chewing increases the salivary flow rate but not the protein concentration and alpha amylase levels. [24] Their finding is consistent with the results of this study and our preliminary study.

Arhakis et al in their study concluded that the amylase level in stimulated saliva (chewing sugar-free gum) is 1.5 times more that its level at rest, while salivary flow increases 6.3 times. [26] The most interesting fact was that among all individuals, mobile users and non- mobile users, the amylase activity is not equal on both sides and often had 2 to 3 times difference with the other side.

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Physiologically and anatomically, the parotid glands of a healthy person should have approximately equal amount of secretion on both sides. It seems that the difference in salivary flow of both sides is the main cause of difference in amylase activity. After collecting 50 salivary samples, the dominant chewing side could be determined. This observation strengthened the hypothesis. Further study in this area is recommended.

In all studies, fluctuation in salivary flow rate was simultaneous with change in salivary amylase concentration. The exact cause of change in amylase concentration is unknown. The most likely mechanism seems to be that chewing stimulates parotid flow through mechanical receptors located in periodontal ligament, gingival tissues and tongue.[24] The mechanism of protein secretion controlled by the nervous system is not exactly clear. Sympathetic and parasympathetic systems are respectively in charge of secretion of salivary proteins and salivary flow. During salivary reflexes, both mechanisms play important roles. Parasympathetic system leads to an increase in flow rate. It also has a stimulating effect on the secretion of non- storage granules. Consequently, it causes an increase in secretion and synthesis of proteins. But high levels of water output neutralize increased protein synthesis. [24]

Enlargement and increased gland activity on dominant chewing side are probably another mechanism that can increase salivary flow on that side. In short, due to increased muscle movement on dominant chewing side, parotid gland saliva is more affected by mechanical stimulations on that side and causes increased salivary flow on the same side. In addition, parotid gland duct cells are responsible for the reabsorption of saliva into circulation. [27] On dominant chewing side, these cells have no opportunity to reabsorb water. As a result thinner saliva is released in the oral cavity. In contrary, more chance of reabsorption provides thicker saliva on the other side.

Conclusion

Handheld mobile phone was not effective on parotid amylase enzyme activity whereas chewing was effective on parotid amylase enzyme activity.

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Authors' contribution

The study was designed by Hadi Mortazavi.The study data were collected by Hadi Mortazavi and Seyed Ali Asghar Sefidgar. Analysis and interpretation of data, drafting of the manuscript, and critical revision of the manuscript for important intellectual content were pre-formed by Ali Bijani and Nafiseh Ghasemi. Study supervision was performed by Neda Babaee, Hamed kazemi and Mehdi Pouramir**.**

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