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SHORT COMMUNICATION article

Effect of smoking duration on salivary α-amylase in Libyan cigarette smokers

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Abstract: Tobacco smoking negatively affects the quality of saliva and affects many biological parameters including salivary α-amylase enzyme activity. Salivary α-amylase enzyme is essential for the catabolism of carbohydrates. Forty- five healthy male volunteers aged between 18 and 69 years (40.0±15.0) were divided into two groups, namely, the control group (n=21) and the cigarette smoker group (n=24). The effect of smoking on salivary α-amylase enzyme activity depends on the number of smoked cigarettes per day and the type of cigarettes as well as the period for how long the person smoking tobacco cigarettes was investigated. Different methods were used to analyze the activity of salivary α-amylase enzyme including the dinitrosalicylic acid method (standard colorimetric method). The results showed a significant increase ($P<0.05$) in salivary α-amylase activity in Libyan smokers compared to the non-smokers in the morning and night, also, the findings showed a significant decline in salivary α-amylase activity during the increasing smoking period. The activity of salivary α-amylase in the young-age smokers group increases in morning and night compared to the non-smokers group. There is no change in the activity of salivary α-amylase in the middle and older adult groups in the morning and night compared to young smokers' group. In conclusion, smoking for a long period time more than 20 cigarettes per day decreases the activity of salivary α-amylase in Libyan subjects.

Introduction

Saliva is an extracellular fluid produced by salivary glands and secreted in the mouth through salivary ducts. This fluid is secreted by the major salivary glands such as parotid, submandibular, sublingual glands, and minor salivary glands, which are too small and found in the lips, roof of the mouth, inside the cheeks, nose, sinuses, and larynx [1]. It has several biological functions, primarily, it lubricates the mouth and throat as well as keeps them clean and comfortable. Moistening the food and changing it to a liquid or semisolid mass that can be tasted and swallowed more easily. Saliva has anti-microbial activity; thus, it protects the oral cavity and gum from diseases. It reduces tooth decay by cleaning the tooth surface and removing food debris, dead cells, and bacteria [2]. Saliva maintains the pH of the mouth by neutralizing the acidity and keeping it at a normal range (pH 6.4-7.4) [3, 4]. Saliva contains a digestive enzyme amylase, which breaks down carbohydrates into simpler compounds. Saliva

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contains 99.0% water and 01.0% different low molecular weight substances, hormones, antibodies, antimicrobial ingredients, growth factors and enzymes such as amylase and lipase [2, 4-6]. α-Amylase (1,4-α-D-glucan glucanohydrolase, EC 3.2.1.1) is an endoglycosidase that catalyzes the random endohydrolysis of 1,4-α-glucosidic linkages in the starch, glycogen and other glucose polymers. In humans, α-amylase enzyme is secreted by the salivary gland and pancreas and is present in the saliva and serum [7, 8]. Most starches contain 20.0% to 28.0% amylose and 72.0%-80.0% amylopectin. Amylase hydrolyses amylopectin significantly faster than amylose. α-amylase is a calcium-requiring metalloenzyme, for full enzymatic activity and stability of the enzyme, at least one calcium atom is tightly bound per protein molecule. The crystal structure of the α-amylase enzyme showed that the allosteric site of the enzyme binds with a chloride ion. This ion enhances the enzyme activity and structure stability [9]. The optimal pH for amylase activity is around neutrality, pH 7.0, but can shift in the absence of chloride. Tobacco consumption causes cardiovascular pathologies, pleuropneumonias, damage to the oral cavity, and cancers [10]. In addition, tobacco consumption modifies several biological parameters, including α-amylase enzyme activity [11]. Several reports have shown the harmful effects of numerous tobacco components [12]. β-nicotine addicts in Libya get their daily dose through different smoking habits, including smoking cigarettes, water pipes (Narghile, Hookah, Shisha) and inhaling snuff, known locally as Naffa. In addition to nicotine, heavy metals are also contained in tobacco products [13]. Cigarette smoke contains many organic compounds, including aromatic amines, nitrosamines, oxidants such as oxygen-free radicals, and volatile aldehydes (acrolein and crotonaldehyde) [14]. The main alkaloid of tobacco is nicotine, which is responsible for its addictive effect. It is easily absorbed from tobacco smoke; in regular smokers, its concentration rises over 6-8 hours during the day. 70.0%-80.0% of nicotine is metabolized to cotinine which is the main metabolite of nicotine. Its half-life in the body is 12-20 hours and is longer than that of nicotine (3-4 hours). Nicotine has widely been used as a specific biomarker of tobacco exposure. Cotinine is suitable for assessment of doses over long periods (weeks or months, in hair or nails) or short periods (from one to ten days, in urine, plasma, or saliva) [15]. Several studies carried out on tobacco dependence's impact on the activity of salivary α-amylase resulted in divergent viewpoints. Thus, smoking tobacco increases the value of α-amylase enzyme activity in saliva [16]. On the contrary, other authors reported that smoking tobacco does not affect the value of serum and salivary α-amylase activity [17-19]. Nevertheless, investigations on the influence of smokeless tobacco consumption on the enzyme α-amylase activity are limited. Likewise, reports on α-amylase activities among cigarette smokers have remained scarce in Libyan society [20]. Thus, this study aimed to investigate how smoking duration affects the α-amylase enzyme activity in the saliva of Libyan cigarette smokers.

Materials and methods

Volunteers: Forty-five healthy Libyan male adult subjects aged between 18 and 69 years (40.0±15.0 years) were divided into two groups (**Table 1**): the control group (n=21) and the cigarette smoker group (n=24). Volunteers with a history of systemic diseases such as diabetes mellitus, hypertension and kidney diseases even late elderly individuals over 75 years old were excluded from the study. All the healthy volunteers were from Tripoli city and the study was carried out for two months: August 2022 and September 2022.

Sample collection: All the volunteers were given a self-designed questionnaire which was validated by health professionals at the University of Tripoli. The questionnaire included questions about age, smoking habits, and written instructions on how the saliva sample can be collected. Unstimulated whole saliva samples were collected from volunteers in sterile containers using a draining method in which the subject is made to sit quietly with the head bent down and the mouth open to allow the saliva to drip passively from the lower lip into the graduated

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sterile tubes [21]. An oral consent was obtained from each volunteer to participate in the study. The volunteers were instructed verbally to collect two saliva samples; the morning sample was given immediately after waking up and at night sample was given finally before going to bed, one hour after finishing the last cigarette or last meal. All the samples were frozen immediately after collection and analyzed within 24-72 hours of the collection.

Sample analysis: The activity of the enzyme salivary α-amylase was determined by colorimetric method using 3,5-dinitro salicylic acid (DNS) as has previously been published by Miller [22].

Table 1: Distribution of the participant's smokers

Age (years)	Group	Frequency	Mean age±S.D.
18-30	Cigarette	08	23.6±2.6
	Control	10	24.2±2.0
31-50	Cigarette	09	42.0±6.0
	Control	06	40.5±5.0
51-69	Cigarette	07	58.0±6.2
	Control	05	58.0±6.3

Statistical analysis: All the analysis was done by using IBM SPSS version 21 program. The mean ± S.D. of the data was calculated by using descriptive analysis. A comparative analysis was done by using an independent sample of the Student *t*-test. The correlations between the different parameters were verified by the Pearson correlation coefficient test. A P value of <0.05 was considered significant.

Results and discussion

Salivary α-amylase activity in morning and night for the volunteers: The statistical analysis of the data has revealed a marked increase of the salivary α-amylase activity for all the cigarette smokers from morning to night, which explains the stimulation of sympathetic nervous system activity during the daytime [16, 23, 24]. These results were significant at p<0.05 (**Table 2**). The baseline of salivary α-amylase activity in the morning was higher in smokers compared with non-smokers. This finding proves that nicotine in tobacco cigarettes stimulates sympathetic nervous system activity [25, 26].

Table 2: Salivary α-amylase activity in the morning and at night as mean ± S.D.

subjects	**α-amylase activity (u/ml)**	
time	**morning**	**night**
non-smokers	22.8±4.6	23.8±4.78
smokers	25.6±3.2	27.16±2.01
P value	**< 0.05**	**< 0.05**

Salivary α-amylase activity in the morning and night for different age groups: The subjects were divided into three age groups: young adults (18-30 years), middle-aged adults (31-50 years), and older adults (51-70 years). Thus, the activity of salivary α-amylase of young-age group smokers was higher in the morning and at night with both values being nearly equal (27.50±2.00 and 27.85±1.30) compared to the control value (19.75±4.11 and 20.73±4.77), respectively, however, the difference was found to be significant (**Tables 3, 4**). The other two groups (middle-aged and older adults) showed an increase in salivary α-amylase activity (except for the morning value

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of the middle-aged group) but the difference was not statistically significant. There was no significant difference in the salivary α-amylase activity among smokers in the morning and night by independent Student *t*-test performed for smoker age groups in the morning between the young-age group and the middle-aged group. The statistical analysis between the young-age group (18-30) and older adults group (51-70) was found to be p=0.067 and p=0.499, respectively, between the middle-aged group (31-50) and older adults' group (51- 70). These results showed no significant difference in the middle and older adult age groups but the high activity was in the young age group.

Table 3: Salivary α-amylase activity in different age groups in the morning

Groups	15-30 years	31-50 years	51-70 years
Control	19.75±4.1	26.78±1.7	23.97±4.1
Smokers	27.5±2.0	25.19±2.6	23.95±4.0
P value	**< 0.01**	**> 0.1**	**> 0.1**

Data expressed as mean±S.D. of enzyme activity (u/ml).

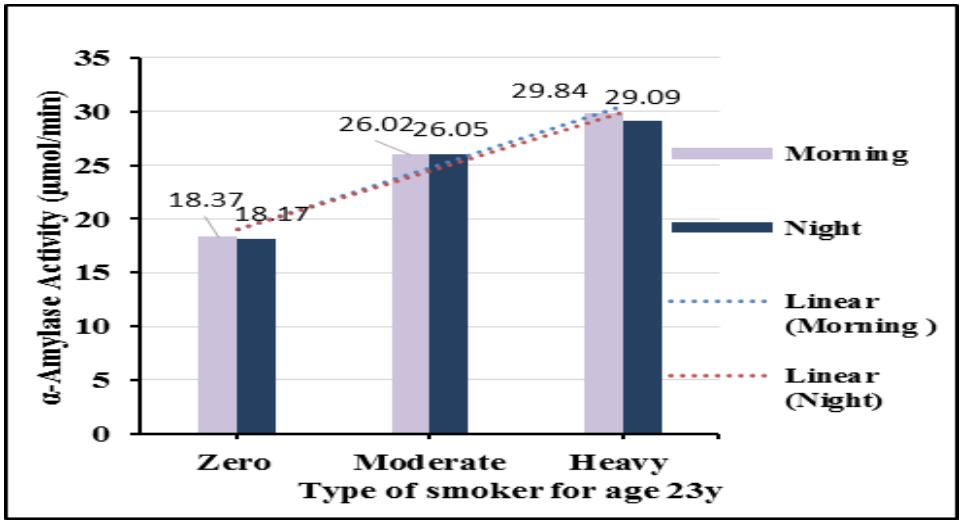
Table 4: Salivary α-amylase activity in different age groups in the night

Groups	15-30 years	31-50 years	51-70 years
Control	20.73 ± 4.8	27.31 ± 2.4	25.6 ± 3.1
Night	27.85 ± 1.3	27.61 ± 1.81	25.79 ± 2.3
P value	**< 0.01**	**> 0.1**	**> 0.1**

Data expressed as mean±S.D. of enzyme activity (u/ml)

Effect of tobacco-smoking period and the number of cigarettes consumed per day on salivary α-amylase activity: Pearson product correlation of salivary α-amylase and tobacco-smoking period was found to be moderately negative and statistically significant for a morning (r= - 0.432, p 0.05) and a night (r= - 0.406, p<0.05). The results indicated that the increase in the smoking period would decrease salivary α-amylase activity (**Figures 1** and **2**).

Figure 1: Salivary α-amylase activity at age 23 years Smoking period for the participants (4-6 yeas)



zero: non-smokers, 5.0 ± 0.8

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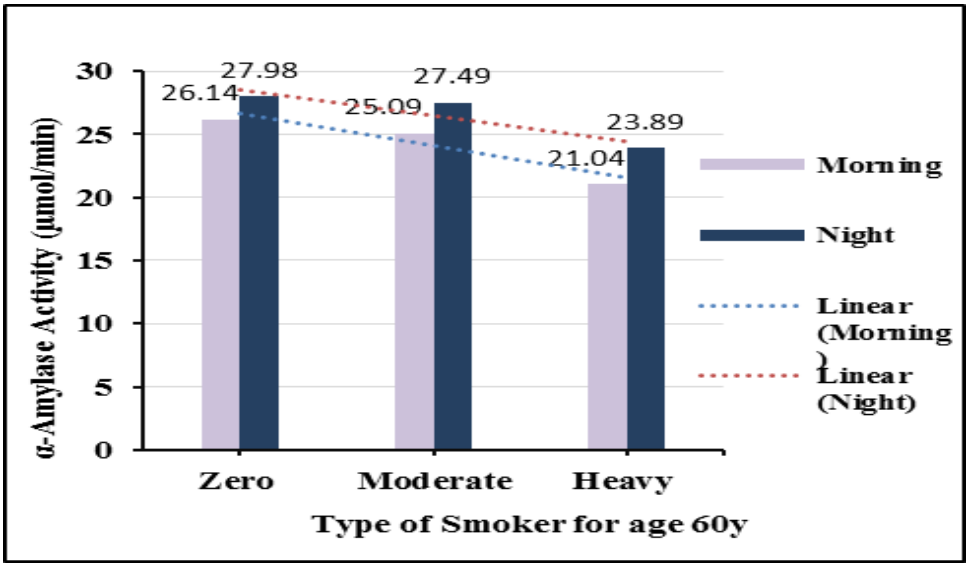
Figure 2: Salivary α-amylase activity at age 60 years Smoking period for the participants (41-46 years)



zero: non-smokers, 44.0±2.0

Thus, the number of cigarettes and smoking period have a significant effect on the activity of salivary α-amylase. Young volunteers who consumed 10-20 cigarettes per day for five years showed a marked increase in the salivary α-amylase enzyme activity as well as heavy smokers who consumed >20 cigarettes per day for five years. From **Figure 2**, the results showed a significant decrease in the α-amylase activity with moderate and heavy smokers consuming more than 20 cigarettes per day for 44 years. In general, acute and long-term exposure to cigarette smoke, in either active or passive smokers, leads to acute and chronic changes in the balance of the autonomic nervous system, resulting in sympathetic predominance [27]. However, it must be considered that the amount of saliva decreases significantly with the duration of smoking and the increasing age of smokers, and long-term smoking significantly reduces the salivary flow rate [28]. It also is known that an unstimulated salivary flow rate is used as an accurate method to evaluate salivary gland function [28] which means that a reduced salivary flow rate could indicate salivary gland dysfunction. This detrimental effect of long-term smoking on the salivary glands could explain the decrease in salivary α-amylase in individuals who smoked for 44 years. However, the present study was carried out in a small size sample in a limited area in Libya, further studies with bigger sample sizes and different regions in Libya are highly recommended to confirm the present findings.

Conclusion: This study demonstrated that tobacco use affects salivary α-amylase activity by direct and indirect effects. The activity of salivary α-amylase in young age smokers increases in the morning and the night. There is no change in salivary α-amylase enzyme activity in the middle and older adult groups in the morning and the night. Smoking for a long period of time more than 20 cigarettes per day decreases the activity of salivary α-amylase.

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Ethical issues: Including plagiarism, informed consent, data fabrication or falsification, and double publication or submission were completely observed by the authors.
Data availability statement: The raw data that support the findings of this article are available from the corresponding author upon reasonable request.
Author declarations: The authors confirm that all relevant ethical guidelines have been followed and any necessary IRB and/or ethics committee approvals have been obtained.

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