World Journal of Advanced Research and Reviews
eISSN: 2581-9615 CODEN (USA): WJARAI
Cross Ref DOI: 10.30574/wjarr
Journal homepage: https://wjarr.com/

(RESEARCH ARTICLE)

Check for updates

The effect of smoking on amylase enzyme activity in saliva

Friska Miani Silitonga * and Annisa Sherina Winarya

Faculty of Dental Medicine, Airlangga University, Surabaya, Indonesia.

World Journal of Advanced Research and Reviews, 2024, 24(02), 350–354

Publication history: Received 22 September 2024; revised on 31 October 2024; accepted on 02 November 2024

Article DOI: https://doi.org/10.30574/wjarr.2024.24.2.3331

Abstract

Smoking has become a common habit in Indonesia. If done regularly, smoking is known to cause problems in the oral cavity. When smoking, cigarette smoke will enter the oral cavity and affect the activity of the salivary amylase enzyme. The journal search used in this review was conducted on several databases, such as PubMed, ScienceDirect, and Google with a range of years between 2013 and 2023. A total of 14 studies from various global populations were included in this review. Thirteen studies showed an association between smoking and alpha-amylase enzyme activity, while one study did not show an association between smoking and amylase enzyme. Of the 13 studies that showed an association, ten studies showed a negative association, while the remaining three studies showed a positive association. The effect of cigarettes on amylase activity in saliva may arise due to several factors, including the toxic effects of cigarettes on the salivary glands, mechanical and chemical stimulation of cigarettes on the mucosa, smoking period, oxidative stress index, and salivary pH.

Keywords: Smoker; Alpha-amylase enzyme activity; Saliva; Non-smoker

1. Introduction

Nowadays, smoking has become a common habit in Indonesia. There was an increase in the prevalence of smoking in the population aged 10 years from 28.8% in 2013 to 29.3% in 2018. This shows that smoking is not only a problem among adults, but also among children and adolescents. The distribution of the population aged ≥ 10 years who smoke every day is highest in Riau Islands Province (27.2%), followed by West Java Province and Bengkulu (27.1%). The lowest distribution was in Papua (16.3%), Bali (18%), and East Nusa Tenggara (19.7%). In Indonesia, the highest prevalence of daily smokers is in the age range of 25-64 (30.7% - 32.2%). Meanwhile, the prevalence of smokers in the age range of 15-24 years reached 18.6% [1].

Saliva is a remarkable fluid as it is the first biological fluid to encounter inhaled cigarette gas extracts and water-soluble cigarette extracts. Salivary biomarkers have gained popularity in stress research as they have proven superior in testing biomarkers in blood [2]. α-amylase is a small heterogeneous enzyme with a molecular weight of 50-55 KDa that requires calcium and chloride ions to perform its activity. α-amylase is important in physiological starch digestion, it breaks down large insoluble starch molecules into smaller soluble molecules (oligosaccharides and then into maltose and glucose) by hydrolyzing the α-1,4 bonds that bind the monosaccharides. In human physiology, salivary and pancreatic amylases are α-amylases produced in the pancreas and salivary glands in different forms, namely salivary type (S-type) and pancreatic type (P-type) [3].

If done regularly, smoking is known to cause problems in the oral cavity. When smoking, cigarette smoke will enter the oral cavity and affect the activity of the salivary amylase enzyme [4]. Cigarettes contain more than 4000 different chemicals and 400 of them have been shown to be carcinogenic. These carcinogenic ingredients include aromatic amines, nitrosamines, oxidants such as free radical oxygen, and high concentrations of toxic volatile aldehydes including

* Corresponding author: Friska Miani Silitonga

acrolein, crotonaldehyde (α,β-unsaturated aldehydes), and acetaldehyde (saturated aldehyde). These ingredients are likely to be the main culprits in the damage of several molecules exposed to cigarettes [5]. Cigarette smoke can have an impact on reducing the volume of salivary secretions, causing a decrease in bicarbonate secretion in saliva which can have an impact on reducing salivary pH. Changes in pH in saliva then determine the activity of the amylase enzyme in saliva [4]. Amylase works optimally in the pH range of 6.8-7. pH affects the work of enzymes by changing the structure of the enzyme so that it affects the speed of activity of an enzyme [6].

2. Methods

The journal search used in this review was conducted on several databases, such as PubMed, ScienceDirect, and Google with a range of years between 2013 and 2023. The search terms used around "smokers" and "non-smokers" were combined with terms relating to "changes in alpha-amylase enzyme activity" and the search was completed with a manual search reference list of each paper identified as relevant. The journals used in this review consisted of English and Indonesian language journals, where the studies used in the journals consisted of retrospective cohort studies and Cross Sectional studies. A review of the associations reported in these studies was conducted, with a synthesis of potential explanations.

3. Results

A total of 14 studies from various global populations were included in this review. A summary of these studies is presented in Table 1. Most of the studies used a cohort study design (n=10). Only four studies used a cross-sectional study design. Among the studies included in the review, 13 studies showed an association between smoking and alpha-amylase enzyme activity, while one study did not show an association between smoking and amylase enzyme. Of the 13 studies that showed an association, ten studies showed a negative association, while the remaining three studies showed a positive association. A negative relationship indicates a decrease in alpha-amylase enzyme activity due to cigarette use, while a positive relationship indicates an increase in alpha-amylase enzyme activity due to cigarette use.

Table 1 A summary of these studies

Positive	**Negative**	**Unrelated**
Hasan & Jabir, 2017 [7]	Jebril *et* al., 2023 [5]	Enemchukwu *et al*., 2013 [17]
Singh *et al.,* 2018 [8]	Zainulabdeen & Alak, 2014 [3]	
Ahmadi-Motamayel *et al*., 2016 [9]	Yordanova, 2019 [10]	
	Ponnaiyan *et al*., 2019 [11]	
	Sitompul *et al*., 2022 [12]	
	Syauqy & Fitri, 2017 [4]	
	A'yun *et al*., 2022 [13]	
	Grover *et al*., 2016 [14]	
	Ahmadi-Motamayel *et al*., 2016 [15]	
	Challap *et al*., 2019 [16]	

4. Discussion

Several studies involved in this review have shown some association between smoking and salivary amylase enzyme activity. The relationship and possible explanations for the relationship are discussed below.

4.1. Toxic effects on salivary glands

Jebril *et* al., 2023 found the Michaelis constant (Km) values in non-smokers and smokers in this study were 0.50 ± 0.02 mM and 1.12 ± 0.08 mM, respectively. The Km value of the enzyme of the non-smoker subject was lower compared to the Km value of the smoker subject. A low Km value indicates a high affinity of the enzyme to the substrate. The increased Km value in the smoker group was due to structural changes in the enzyme induced by CS, which may partially

define the protein modification caused by CS, or due to the low accessibility of the substrate to the active site of salivary α-amylase. The Vmax of salivary α-amylase for nonsmokers was 31.25±8.24 nmol/min/mg protein and for smokers 18.10±4.04 nmol/min/mg protein. The increase in Km and decrease in Vmax values of smoking subjects occurred due to less availability of substrate to the active site of the enzyme due to chemical bonding, diffusion restriction, and confinement of enzyme molecules with polymer support. In addition, the additional specificity constant Ks value for α-amylase of non-smoker saliva (62.50±15.21 ml/min/mg protein) again confirmed that α-amylase of non-smoker saliva is higher and more specific for starch compared to α-amylase of smoker saliva (Ks = 16.50±6.41 ml/min/mg protein) [5].

From the results of the study, it can be observed that smokers showed a decrease in serum S-type isoamylase which correlated well with a significant decrease in salivary amylase activity. This could be because acute administration of nicotine to non-smokers is associated with increased salivary amylase activity. Thus, the increase in salivary amylase in non-smoking subjects could be the result of decreased metabolic clearance of amylase, pancreatitis, or parotitis. Parotitis is a salivary disease associated with increased S-type isoamylase. Parotitis is usually caused by trauma, stress, or surgery to the salivary glands, radiation to the neck region involving the parotid glands, and subsequent duct obstruction, or stones in the salivary ducts. Evidence suggests a measure of asymptomatic pancreatic, salivary and parotid gland dysfunction among smokers. Determination of salivary α-amylase activity can be useful in detecting parotid/salivary gland disease at an early stage. Overall, smokers have a considerable risk of developing the disease as assessed by the significant increase in salivary α-amylase activity values [5].

4.2. Cigarette mechanical and chemical stimulation of the mucosa

Salivary a-amylase is a digestive enzyme that plays a role in carbohydrate metabolism. The parotid gland is the major salivary gland, synthesizing and secreting alpha-amylase (70-80% of the total amount of enzyme). Amylase activity in saliva varies widely between 15,000 and 800,000 U/L. This occurs due to reverse water absorption in the grooved canal and osmotic transport from the mating canal. It is considered a marker for serous cell function. Its elevated value is a typical example of the influence of the sympathetic nervous system (stress or beta mimetic stimulation) on salivation. However, this effect is depleted by the duration of smoking and the number of cigarettes smoked per day [10].

Changes in all biochemical parameters included in this study were assessed by comparing the blood values of the nonsmoker group and the smoker group, a reduction in the level of amylase activity (211.074±157.224 vs. 345.450±236.2134, $p<0.05$) and amylase specific activity (26.111±12.814 vs. 55.50±21.045, $p<0.001$) was observed. The reduction in serum enzymatic activity of narghile smokers is most likely due to the interaction between smoke aldehydes and the -SH group of the amylase molecule [3].

4.3. Smoking period

The results of a study conducted by Ponnaiyan et al., 2019 revealed that amylase activity decreased in patients with the highest ETS exposure (11-15 years) compared to the least exposure (1-5 years) which was statistically significant ($P < 0.05$). This lower salivary amylase activity due to tobacco smoke exposure only occurred in mothers, but not infants. The low amylase levels in subjects with high ETS exposure could be due to inhibition of salivary amylase by cigarette smoke due to the interaction between aldehydes in cigarettes and the SH-group of the enzyme molecule [11].

The same results were also found in Syauqy & Fitri's research (2017). In the oral cavity, which is the first place exposed to cigarette smoke when smoked by smokers, various oral and dental diseases can occur. These diseases extend from poor oral hygiene, tooth loss, dental caries, bad breath, smoker's melanosis, smoker's palate, periodontitis, precancerous lesions such as leukoplakia and even cancer. Increased daily tobacco use reduces salivary secretion and its bicarbonate content. A cigarette consumed per day requires at least 5 cigarettes and a smoking period of at least 1 year to achieve this effect. Based on the results of the study, this effect is due to the disruption of bicarbonate secretion in saliva, and the reduction of bicarbonate lowers the pH of saliva, which decreases the activity of the saliva ptialin enzyme. In addition, in this study, based on statistical analysis using the Fisher test, it was found that there was a relationship between the length of smoking of students and the activity of ptyalin enzyme (p-value 0.000) [4].

4.3.1. Oxidative stress index

Hasan & Jabir (2017) showed an increase in serum amylase activity in all three smoking groups compared to the control group, but this increase was not significant ($P>0.05$). The observed increase in amylase-specific activity in the case of the smoking groups ((CS) & (CS&N) groups) appears to be a result of increased expression of its gene. Further studies are needed to substantiate these conclusions, with such further studies using a larger sample size and considering the total time of exposure to smoke (i.e. number of cigarette smoke/day). The period a person smokes, age, and gender act

as factors that may influence the results obtained and the risk of disease development Stress and psychological stress. Such factors may be associated with higher levels of oxidative damage and are considered strong contributors to oxidative damage, through the production of free radicals in the autooxidation of catecholamines. Oxidative stress reflects a state of cellular imbalance, in which the production of ROS exceeds the antioxidant mechanisms that neutralize ROS, resulting in oxidative damage to nearby molecules, such as DNA, RNA, and lipids [7].

Based on the positive correlation between salivary α-amylase & OSI found in the smoker group (CS&N), researchers observed that saliva is more affected by cigarettes than serum. This is because saliva is the first body fluid to encounter inhaled smoke and a synergistic and deleterious interaction occurs between smoking and saliva. While in serum, the (CS&N) group was the most affected group than the others. This can be explained as a combined effect of the different composition of the cigarette products and the different temperatures involved in these types of cigarettes. Based on the fact that amylase activity increased upon psychological stress in all groups of smokers in this study, it can be proposed that instead of helping people relax, smoking actually increases anxiety and tension. Thus, although smoking reduces symptoms of stress, which are similar to feelings of anxiety, it does not reduce anxiety or address its underlying causes [7].

4.3.2. pH saliva

Research conducted by Sitompul *et al* (2022) involved 50 students, 40 of whom smoked more than 10 cigarettes per day. As a result, it was found that the average salivary pH of smoker students was around 6.3, more acidic than the control pH of 6.8. In addition, the average salivary amylase activity in smokers was 68% lower than the control, which was 75.5%. These two things can be related because as is well known, the optimum pH of amylase is in the range of 6.8-7 so that when the pH decreases, the activity of the salivary amylase enzyme also decreases. Similar effects also appear in e-cigarette users [12]. Based on research by A'yun, Hidayati, & Kurniawan (2021), of the 30 respondents who used e-cigarettes, 22 of them showed a salivary pH that tended to be more acidic. Conversely, in 30 non-smoking respondents, 22 of them had a normal pH. The average pH of e-cigarette smokers is 6.7 and the average pH of non-smokers is 7.4. Cigarettes that spread continuously in the oral cavity cause sensitivity and changes in taste receptors, causing suppression of the salivary reflex. This change has an impact on the salivary flow rate which has a major impact on the pH value of saliva. Salivary pH in smokers is lower than non-smokers due to a decrease in the salivary buffering response caused by the effects of nicotine contained in cigarettes. The salivary rate decreases gradually due to exposure to nicotine [13].

The impact of smoking on salivary pH is also proven by research from Grover et al (2016) and Ahmadi-Motamayel et al (2016). Both studies show the same results, namely long-term cigarette consumption can cause significant changes in salivary pH to become more acidic [14][15]. Research by Challap et al (2019) also showed a decrease in the average level of salivary pH in tobacco smokers compared to the non-smoker group although it was not significant. This may be because the pH meter used was not sensitive enough to detect small differences in salivary pH [16].

Research by Singh et al (2018) shows that the level of α-amylase in smokers is higher than non-smokers. The α-amylase level in the smoker group was 206.25 U/mL while in the non-smoker group it was 169.85 U/mL. Between these two groups no significant results were found ($p > 0.05$) [8]. Similar results were also shown by research from Ahmadi-Motamayel (2016), the average α-amylase level in the smoker group was 214.10 U/mL while the average α-amylase level in the non-smoker group was 144.55 U/ml with insignificant differences ($p = 0.265$). However, these two studies did not mention the exact thing that caused the α-amylase level in the smoker group to increase compared to the non-smoker group [9].

5. Conclusion

Based on the research that has been conducted, there are differences in the results obtained from previous studies regarding the effect of smoking on amylase enzyme activity in saliva. Three studies showed positive effects, ten studies showed negative effects, and one study found no relationship between smoking and amylase enzyme activity. The effect of cigarettes on amylase activity in saliva may arise due to several factors, including the toxic effects of cigarettes on the salivary glands, mechanical and chemical stimulation of cigarettes on the mucosa, smoking period, oxidative stress index, and salivary pH. Further research is needed to determine the exact relationship between smoking and amylase enzyme activity in saliva.

Compliance with ethical standards

Acknowledgments

We thank the librarian of the University of Airlangga for the help with the search strategy and full-text provision.

Disclosure of conflict of interest

The author(s) declare that they have no Conflict of Interests.

References

[1] Sitohang, V., & Budijanto, D. Indonesia health profile 2013. Jakarta: Indonesian Ministry of Health; 2013.

[2] Yi, T.C., & Moochhala, S. Mini-Review Article - Current opinion on salivary biomarkers as a measurement for stress and fatigue. The Open Biomarkers Journal. 2013; 6: 9-14. doi:10.2174/1875318301306010009.

[3] Zainulabdeen, J. A., & Alak, S. A. Effects of Smoking on Proteins Level and Alpha Amylase Activity in Sera of Iraqi Narghile Smokers. Iraqi Academic Scientific Journals. 2014; 25(3): 33-40.

[4] Syauqy, A., & Fitri, A. D. The Effect of Smoking on Salivary Ph and Ptialin Enzyme Activity in Students of the Faculty of Medicine and Health Sciences, Jambi University. Jambi Medical Journal. 2017; 5(1): 29-40.

[5] Jebril A.O., Abuskhuna S.M., Alzorqani A.M., Rhbeida O. A. Effect of smoking duration on salivary α-amylase in Libyan cigarette smokers. Mediterr J Pharm Sci. 2023; 3(2): 51–58.

[6] Murray, R.K. Biokimia harper. 27th ed. Jakarta : EGC; 2012.

[7] Hasan, H. R., & Jabir, R. J. Salivary and serum α-amylase activity and their correlations with oxidative stress index among different Iraqi smokers groups. MOJ Addict Med Ther. 2017; 4(1): 224-230.

[8] Singh, Satvinder & Sharma, Mamta & Rohilla, Nitika & Salgotra, Varun & Kumar, Varun & Sharma, Rahul. Assessment of Salivary Catalase, a-Amylase, and Cotinine Levels in Chronic Smokers: A Comparative Study. The Journal of Contemporary Dental Practice. 2018; 19: 253-256. doi: 10.5005/jp-journals-10024-2247.

[9] Ahmadi-Motamayel, F., Falsafi, P., Goodarzi, M. T., & Poorolajal, J. Comparison of Salivary pH, Buffering Capacity and Alkaline Phosphatase in Smokers and Healthy Non-Smokers: Retrospective cohort study. Sultan Qaboos University Medical Journal. 2016; 16(3): 317–321. doi:10.18295/squmj.2016.16.03.009

[10] Yordanova, M., & Shopov. N. The Effect of Smoking on Salivary Parameters. Family Medicine and Primary Care : Open Access. 2019; 3: 134. https://doi.org/10.29011/JFOA-134/100034

[11] Ponnaiyan, D., Chillara, P., & Palani, Y. Correlation of environmental tobacco smoke to gingival pigmentation and salivary alpha amylase in young adults. European Journal of Dentistry. 2017; 11(3) 364–369. 10.4103/ejd.ejd_99_17

[12] Sitompul, R., Mashitah, U., Asriati, S., Sigit, R., Kurniawan, Yurnaliza. Effect Of Smoking Behaviour and The BMI On Ph Levels Of Saliva and Salivary Amylase Activity Among Male Students. International Journal of Ecophysiology. 2022; 4(2): 1-8.

[13] A'yun, Quroti. Hidayati, S., Kurniawan, F. Comparative Status of Saliva between Electric Smokers with Non-smokers. International Research Journal of Pharmacy and Medical Sciences. 2021; 5(1): 4-6.

[14] Grover, N., Sharma, J., Sengupta, S., Singh, S., Singh, N., & Kaur, H. Long - term effect of tobacco on unstimulated salivary pH. Journal of Oral and Maxillofacial Pathology. 2016; 20(1): 16-19. doi: 10.4103/0973-029X.180907

[15] Ahmadi-motamayel, F., Falsafi, P., Goodarzi, M. T., & Poorolajal, J. Evaluation of salivary catalase , vitamin C , and alpha- amylase in smokers and non-smokers : a retrospective cohort study. Journal of Oral Pathology & Medicine. 2016; 46(5): 377-380. https://doi.org/10.1111/jop.12495

[16] Challap, I. D., Lafta, F. M., & Al-rekabi, A. N. G. Investigation the impact of smoking on salivary pH and the reasons behind smoking. Journal of Physics: Conference Series. 2019; 1295(6). doi: https://doi.org/10.1088/1742-6596/1294/6/062069

[17] Enemchukwu, , B., Ubaoji, K. I., Igwilo, I. O., & Udedi, S. Effects of Temperature, pH and Substrate Concentration on the Kinetics of Salivary Alpha- Amylase Activity among Cigarette Smokers in Awka, Anambra State, Nigeria. The Bioscientist Journal. 2013; 1(1): 108-113