Innovations

Estimation of Salivary Alpha-Amylase and Total Protein among Headphone and Non-Headphone Users-A Comparative Study

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Abstract

Background: *Salivary alpha-amylase (sAA) is a key biomarker for stress and autonomic nervous system activity. Prolonged headphone use has been linked to physiological and psychological stress responses, particularly at high volumes.* ***Aim:*** *The aim of the study is to estimate salivary alpha-amylase and total protein levels among headphone users and non-headphone users.* ***Materials andMethods:*** *The study was conducted in the department of Oral Pathology andMicrobiology after approval from the institutional review board. A total of 60 samplescomprising 2 groups: 30 Non-headphone users - controls (Group 1) and 30 Headphone users - Test (Group 2) of age group between 20 to 30 years and gender-matched individuals were included in thestudy. Unstimulated salivaryalpha-amylase levels were analyzed using the CNPG3 (2-Chloro-4-Nitrophenyl-α-D-Maltotrioside) Method. The samples were collected in the early morning. The samples were centrifugedat 10,000 rpm and we spectrophotometrically analyzed at 540nm.* ***Results:*** *The independent t-test was used for statistical analysis. The mean salivary α-amylase levels of non-headphone and headphone users were calculated for every 30 secs interval i.e. 60 secs, 90 secs, and 120 secs. For 60 secs the mean alpha-amylase levels were 1129.9IU/L for the control group and 1681.0IU/L for the test group. For 90 secs the mean alpha-amylase levels were 702.72IU/L for the control group and 1107.28U/L for the test group. For 120 secs the mean alpha-amylase levels were 510.0IU/L for the control group and 821.20U/L for the test group.* ***Conclusion:*** *It can be concluded that increased alpha-amylase in all three intervals on samples of headphone users when compared to non-headphone users.*

Keywords: *Non-headphone users, headphone users, non-invasive, salivary amylase level, salivary protein level.*

Introduction

Saliva is a vital, multifunctional biological fluid secreted by major and minor salivaryglands, comprising 90% water, 10% enzymes, electrolytes, antimicrobial agents,proteins, hormones, etc. Salivary alpha-amylase (sAA) is an enzyme predominantly secreted by the salivary glands, playing a crucial role in the initial digestion of dietary starches. Beyond its digestive function, sAA has garnered attention as a non-invasive biomarker for sympathetic nervous system activity, particularly in response to psychosocial stressors(1) . Elevated sAA levels have been observed during

acute stress, indicating its sensitivity to sympathetic activation (2).The ubiquitous use of headphones for activities such as music listening, virtual meetings, and entertainment has raised questions about their potential impact on physiological stress markers. While some studies suggest that music delivered via headphones can benefit patients by reducing anxiety and agitation (3), other research indicates that noise exposure from headphones may affect stress levels and even blood pressure (4). However, the relationship between headphone usage and biomarkers like sAA remains underexplored.Total salivary protein concentration is another parameter of interest, as it reflects overall salivary gland function and can be influenced by various physiological and environmental factors. Changes in total protein levels may provide additional insights into the body's response to stress and external stimuli.This comparative study aims to assess the levels of sAA and total salivary protein in individuals who frequently use headphones versus those who do not. By analyzing these biomarkers, we seek to elucidate the physiological implications of regular headphone use and its association with stress-related responses.

Methodology:

The study was approved bythe Institutional Review Board(2024//APDCH). An informed consent was obtained from every individual before sample collection and the patient was verbally informed regarding the procedure. A total of 60 saliva samples were obtained and were grouped into Group 1, non-headphone users (n=30), and Group 2, headphone users (n=30). Demographic datawas collected with age and gender-matched individuals of age between 20 to 30 years with the known usage of headphones (any type and any brand) and for non-headphone users criteria for those who are not using headphones for a minimum of 2 years were included.Subjects under anti-depressive therapy, ENT problems, ear infections, and other stress-related problems were excluded from the study.

Study setting: The samples were collected from the outpatient department of APDCH for headphone users and from the rural belt of Uthiramerur village for non-headphone users. The samples were stored at -20 degrees Celsius. Spectrophotometric analysis of the samples wasdone in the Department of Oral Pathology APDCH.

Sample collection: The saliva sample was collected from the participants after getting proper concern. The individuals were instructed not to eat or drink two hours before saliva collection. About 10 ml of unstimulated saliva was collected from the individuals in a sterile container. The whole saliva was collected and stored at a -20℃ refrigerator. The collected saliva sample is then centrifuged at 10,000 rpm for 10 mins. The supernatant is separated from the sample and stored at -20°C. Salivary total protein and alpha-amylase levels were analyzed using spectrophotometry.

Procedure: Salivary alpha-amylase levels were analyzed using the CNPG3 (2-Chloro-4-Nitrophenyl-α-D-Maltotrioside) method. The standard (S) solution is prepared by serial dilution of reagents (5%,10%,15%,20%). Then 20µL of test samples (T) are added to the 250µL of standard solution in a cuvette. The total protein analysis was done using the biuret method. The prepared test solutions were incubated in a dark room for 20 mins. The final total protein and alpha-amylase levels were analyzed using spectrophotometry at 560 nm.

Results:

Salivary α-amylase and total protein were compared between non-headphone and headphone users. All the data were entered in the structured Excel sheet and an independent sample t-test was performed using SPSS software version 22.0. The mean total protein level among non-headphone users was 2.53g/dl and headphone users were about 0.75g/dl (Table 1), on comparing the control and test groups there was a statistically significant p-value of p=0.000 using a 2-tailed testing method (Table 2). The findings revealed that Group A exhibited significantly higher total protein levels compared to Group B (Fig 1). The mean salivary α-amylase levels of non-headphone and headphone users were calculated for every 30 secs interval i.e. 60 secs, 90 secs, and 120 secs. For 60 secs the mean alpha-amylase levels were 1129.9IU/L for the control group and 1681.0IU/L for the test group. For 90 secs the mean alpha-amylase levels were 702.72IU/L for the control group and 1107.28U/L for the test group. For 120 secs the mean alpha-amylase levels were 510.0IU/L for the control group and 821.20U/L for the test group (Table 3). On comparing the intervals, 60 secs showed increased levels of alpha-amylase followed by a decrease in levels noted at 90 secs and 120 seconds with statistically significant p-values (P=0.00) (Table 4). The results show a declining trend in amylase activity over time in both groups, with Group B consistently exhibiting higher amylase activity compared to Group A at all time points (Fig 2).

Discussion:

This study aimed to compare the salivary alpha-amylase (sAA) activity and total protein concentration between non-headphone users (Group A) and headphone users (Group B). The results indicated that headphone users exhibited significantly higher alpha-amylase activity across all time points (60, 90, and 120 seconds), while total protein levels were notably lower in this group compared to non-headphone users. These findings suggest a possible physiological response to headphone use, potentially involving the autonomic nervous system (ANS) and stress regulation mechanisms.

Salivary alpha-amylase is a well-established biomarker of sympathetic nervous system (SNS) activity, often linked to stress and autonomic activation. It is released in response to psychosocial stress, cognitive workload, and environmental stimuli (Nater & Rohleder, 2009). In this study, the increased sAA levels in Group B suggest that prolonged headphone use might be associated with heightened sympathetic activation, possibly due to the auditory stimulation and cognitive engagement involved in listening activities. Previous studies have shown that exposure to auditory stimuli, particularly at moderate to high volumes, can trigger increased autonomic responses (Takai et al., 2004)(5). This aligns with our findings, where headphone users exhibited consistently higher amylase activity, potentially reflecting a heightened physiological stress response. Furthermore, prolonged activation of the SNS could lead to dysregulation in stress-related biomarkers, necessitating further research on the long-term effects of headphone use on stress physiology(6).

Salivary total protein levels are influenced by salivary gland function, autonomic control, and hydration status. The significantly lower protein levels in Group B (headphone users) could indicate a decrease in overall salivary secretion, possibly due to reduced parasympathetic activity. Studies suggest that chronic SNS activation can inhibit salivary

gland secretion, reducing total protein output (Bosch et al., 2011)(7). One possible explanation is that headphone use may lead to a shift in autonomic balance, favouring sympathetic dominance over parasympathetic activity. This could result in a lower overall saliva flow rate, thereby decreasing total protein concentration. Additionally, factors such as hydration, stress levels, and cognitive workload may further influence this physiological response (Granger et al., 2007)(8).

Takai et al. (2004) demonstrated that acute psychological stress leads to increased salivary alpha-amylase secretion, confirming its role as a sympathetic nervous system (SNS) biomarker(5). Their study suggests that cognitive engagement or sensory input, such as sound exposure, may contribute to increased SNS activity, similar to what we observed in headphone users. van Stegeren et al. (2006) explored the relationship between auditory stimuli, cognitive workload, and salivary biomarkers and found that alpha-amylase secretion increased significantly during tasks requiring focused attention. Their study supports our findings that prolonged headphone use might result in persistent autonomic activation, leading to sustained elevations in sAA levels(9).

Most previous studies focused on acute stressors, while our study suggests that prolonged headphone use could contribute to chronic SNS activation, leading to sustained elevations in sAA and reductions in total protein secretion(10). Studies like those of Bosch et al. (2011) indicate that an imbalance between sympathetic and parasympathetic activity might explain the inverse relationship between sAA and total protein observed in headphone users. While acute increases in sAA are considered adaptive stress responses, chronic elevations could affect oral health, digestion, and overall stress resilience (Allgrove et al., 2008). Our study raises concerns about the potential long-term effects of prolonged headphone use on autonomic function and salivary gland activity(11).

Limitations and Future directions: Investigating whether different types of auditory exposure (e.g., music, speech, white noise) lead to differential effects on sAA and total protein secretion. Longitudinal studies examining the long-term impact of chronic headphone use on stress physiology, cognitive performance, and oral health. Exploring additional biomarkers such as cortisol, immunoglobulins (IgA), and mucins to provide a more comprehensive understanding of autonomic and immune responses in headphone users.

Conclusion:

Compared to previous research, our study highlights a distinct pattern of increased salivary alpha-amylase activity and reduced total protein secretion in headphone users, suggesting that prolonged auditory stimulation may contribute to chronic autonomic nervous system alterations. The findings of this study have important implications for public health and personal listening habits. Chronic exposure to high-intensity sound via headphones may contribute to sustained autonomic activation, which could increase the risk of hypertension, anxiety, sleep disturbances, and cognitive fatigue over time. Given the widespread use of personal audio devices, particularly among students and working professionals, awareness regarding safe listening practices is crucial.

Tables and Graphs:

Table 1:

Independent T-Test					
	Group	N	Mean	Std. Deviation	Std. Error Mean
Total protein estimation	Group A	30	2.53170	1.014178	.185163
	Group B	30	.75977	.265383	.048452

Table 2:

Total protein estimation		F	Sig.	t	df	Sig. (2-tailed)
	Equal variances assumed	52.214	.000	9.258	58	.000
	Equal variances not assumed			9.258	32.953	.000

Table 3:

Independent T-Test					
	Group	N	Mean	Std. Deviation	Std. Error Mean
Activity of amylase - 60s(IU/L)	Group A	30	1129.9744	506.23443	92.42534
	Group B	30	1681.0821	463.93975	84.70342
Activity of amylase - 90s(IU/L)	Group A	30	702.7280	288.82223	52.73148
	Group B	30	1107.2873	296.77238	54.18297
Activity of amylase - 120s(IU/L)	Group A	30	510.0000	213.93055	39.05820
	Group B	30	821.2050	230.24360	42.03654

Table 4:

Activity of Amylase		F	Sig.	t	df	Sig. (2-tailed)
Activity of amylase - 60s(IU/L)	Equal variances assumed	.005	.945	-4.396	58	.000
	Equal variances not assumed			-4.396	57.564	.000
Activity of amylase - 90s(IU/L)	Equal variances assumed	.044	.835	-5.351	58	.000
	Equal variances not assumed			-5.351	57.957	.000
Activity of amylase - 120s(IU/L)	Equal variances assumed	.267	.608	-5.423	58	.000
	Equal variances not assumed			-5.423	57.690	.000

FIG 1:

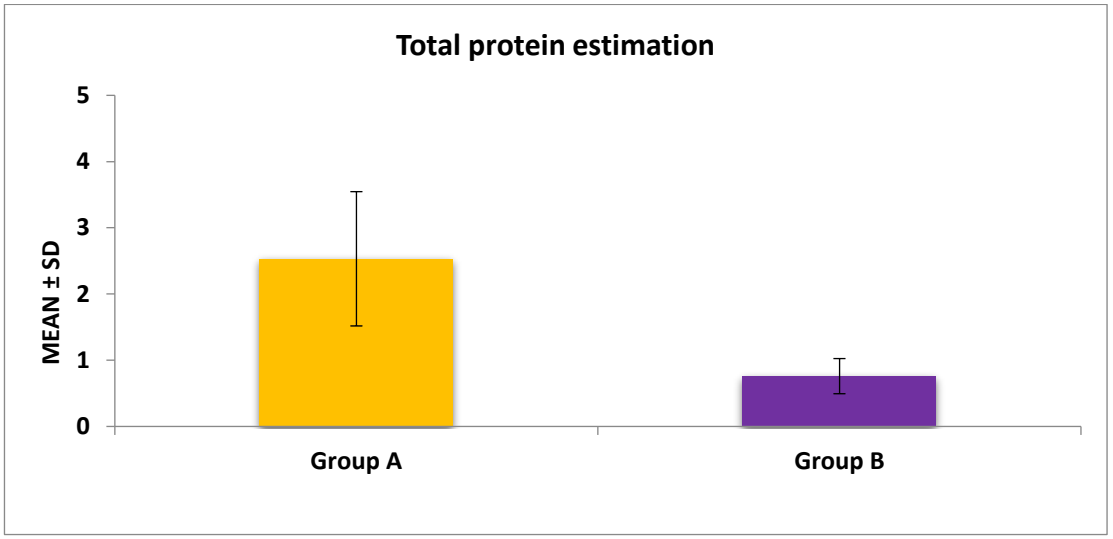

FIG 2:

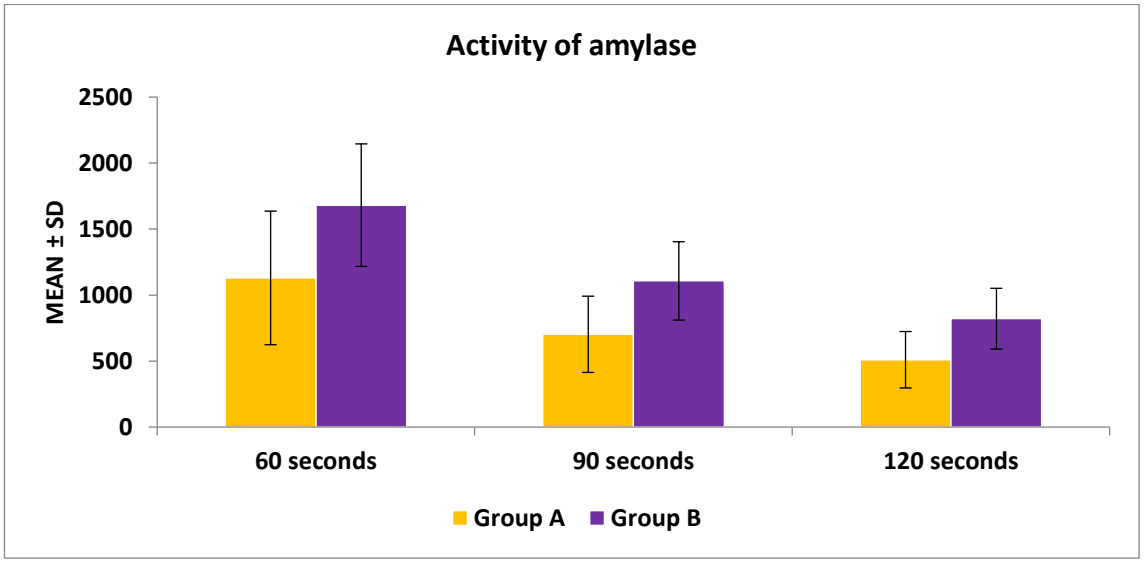

FIG 3:

FIG 5:

FIG 4:

FIG 6:

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