Original Research

Salivary α-amylase as a stress biomarker in mentally and physically disabled individuals during COVID-19 times

Ashish Gaurav[1], Gaganjot Kaur Sharma[2], Ajay Chhabra[1], Sakshi Bamba[3], Sabashish Sahoo[4], Vandana Chhabra[5]

[1]Department of Dentistry, AIIMS, Kalyani, [4]Biochemistry Department, AIIMS, Kalyani, West Bengal, [2]Department of Oral Pathology, Shaheed Kartar Singh Sarabha Dental College, [3]Department of Pediatric and Preventive Dentistry, Shaheed Kartar Singh Sarabha Dental College, Sarabha, Ludhiana, [5]Dr. Harvansh Singh Judge Institute of Dental Sciences and Hospital, Panjab University, Chandigarh, India

Abstract

Background: Stress is faced by every individual in their day-to-day life activities. During pandemic, most people have experienced multiple episodes of depression, anxiety, and insomnia. Several medical and dental problems are reported with stress and the fastest and easiest way to study and investigate is through body fluids, mainly saliva. Hence, we aim to study salivary α amylase in disabled individuals and hypothesize that salivary α amylase could be a reliable stress biomarker in disabled individuals.

Materials and Methods: Cross-sectional study was conducted including 200 individuals grouped into controls (100) and disabled individuals (100). Disabled individuals were further grouped into physically (50) and mentally (50) disabled. Stimulated and unstimulated saliva was collected from all the participants and were investigated for salivary α-amylase by direct substrate method. The enzyme activity was analyzed using a spectrophotometer and the obtained were analyzed statistically.

Result: Salivary α-amylase showed significant difference between controls and disabled group (p = 0.000). Salivary α-amylase was least in the mentally disabled group compared to physically disabled group. Age related comparison of salivary α-amylase was higher in physically disabled whereas gender related comparison showed females in physically disabled group to be more affected than controls.

Conclusion: Saliva, the easy and most researched fluid, is rich in salivary α-amylase. Salivary α-amylase is a potential biomarker to assess stress. Further diagnostic studies are required to know the salivary changes and their influence on individual general health status.

Keywords: Biomarker, pandemic, disabled, salivary α-amylase, stress

INTRODUCTION

Stress is faced by every individual in their day-to-day life activities. During a pandemic, most of the people have experienced multiple episodes of depression, anxiety and insomnia. People with disabilities suffer from multiple barriers, preventing them from accessing essential information and basic care. The World Health Organization (WHO) has provided the criteria to classify physical and intellectual disability based on intelligence quotient levels.[1,2] The pandemic has affected the quality

Access this article online

Quick Response Code:

Website:
https://journals.lww.com/JPAT/

DOI:
10.4103/jomfp.jomfp_462_23

of life (QOL) of disabled individuals. QOL describes an individual's subjective perception of their position in life as evidenced by their physical, psychological and social functioning.[3] The previously reported study has shown an individual living with disability had psychopathologies, such as depression (46.2%), generalised anxiety disorder (48.1%) and insomnia symptoms (71.4%).[4] The QOL of most of the disabled children was decreased in the pandemic.[5]

Several medical and dental problems, such as heart diseases, gastrointestinal (GIT) disorders, certain cancers and oral mucosal diseases, in disabled individuals have been reported to be related to stress in pandemic. The fastest and easiest way to investigate them is through body fluids. Saliva is the most researched and current fluid due to its ease of collection, precision and non-invasiveness. Salivary alpha (α)-amylase is one of the major proteins in saliva with the main function of digestion of carbohydrates and maintaining the mucosal immunity of the oral cavity. Numerous studies showed the association between levels of salivary α-amylase with psychological stress.[6,7] Salivary α-amylase in saliva has been considered a reliable biomarker of stress and anxiety.[6] Changes in the salivary α-amylase due to shift change in the workplace have been observed in hospital nursing professionals. It was shown that the enzyme activity increases in the middle of the work shift and changes in the night shift.[8]

We hypothesise that salivary α-amylase could be a reliable stress biomarker in physically and mentally disabled individuals during a pandemic. To test this hypothesis, this study was conducted to highlight the importance of salivary α-amylase as a stress biomarker in both physically and mentally disabled population and their comparison with normal controls. The study also focused on the factors affecting the levels of salivary α-amylase.

MATERIALS AND METHODS

Study design
The cross-sectional study was conducted for 2 months in the Outpatient Department of All India Institute of Medical Sciences (AIIMS), Kalyani. The study included a total of 200 individuals categorised into two major study groups. Group 1 included 100 individuals as controls and group 2 included 100 disabled individuals who were further divided into physically (50) or mentally (50) disabled. The study population was divided into three age groups: 1) less than 12 years (<12 years), 2) teenagers (13–19 years) and 3) more than 20 years (>20 years). The demographic data are presented in Table 1. In the disabled group, the physically and mentally disabled individuals are separated based on the inclusion criteria as shown in Table 2. The exclusion criteria included

Table 1: Demographic data of the study participants

Study group division	Controls	Disabled	
		physically	Mentally
Age/(M/F)			
<12 years	10 (6M/4F)	2 (2M/0F)	0
13–19 years	21 (9M/12F)	1 (1M/0F)	0
>20 years	69 (35M/34F)	47 (24M/23F)	50 (32M/18F)
Total	100	50	50

Table 2: Inclusion criteria to differentiate physically and mentally disabled study participants

Inclusion criteria	Physically disabled	Mentally disabled
Persons who show noticeable limitation in activities of daily living (ADL) (grade 3)	Hearing impairment Speech impairment Damaged sound emission ability Mobility impairment	Patients suffering from anxiety and depression for more than 3 months.

individuals with severe physical and mental disabilities other than the inclusion criteria. The study was approved by the Indian Council of Medical Research (ICMR) (reference number 2022-07805) for a short-term project and also obtained approval from the research ethics committee of the AIIMS, Kalyani. An informed consent was obtained from all the study participants and/or their guardians after duly explaining the procedure.

Sample collection and statistics
After recording a brief case history, saliva samples both stimulated and unstimulated saliva were collected from each study participant. The unstimulated whole saliva was collected during morning hours between 8.30 and 10 a.m. To limit the circadian variation, study participants were instructed not to eat or drink for at least 45 minutes before the sample collection. The participants were advised to rinse the mouth with plain water, and then, salivary samples (unstimulated and stimulated) were collected by the passive drooling method and spitting method. The whole saliva was collected in the graduated measuring cylinders having a volume capacity of 1 ml. The saliva samples were immediately handed to the laboratory. In the laboratory, all the salivary samples were stored at -20°C and then centrifuged and clear saliva was tested for salivary α-amylase by the alpha-amylase kit, which uses the direct substrate method. It is based on the principle of α-amylase catalysis that hydrolysis 2 chloro-4 nitrophenol salt to chloronitrophenol. The rate of hydrolysis is measured as an increase in absorbance due to the formation of chloronitrophenol, which is proportional to α-amylase activity. The analysis was performed for the α-amylase levels in saliva using a Liquipath alpha-amylase kit. The thawed salivary samples were centrifuged, 20ul was added to α-amylase reagent and enzyme activity was analysed using an ultraviolet (UV) spectrophotometer. The readings were recorded at 1-min, 2-min and 3-min intervals, and the

mean of all three readings was taken as the final reading. The normal range of α-amylase level ranged from 1 to 371 U/ML on a wider range. The data obtained were subjected to statistical analysis using Statistical Package for the Social Sciences (SPSS) version 23.0, and mean, median and standard deviation were calculated. The control and disabled groups were compared for the salivary α-amylase levels using an independent-samples *t*-test, and a *P* value of less than 0.05 was considered significant.

RESULTS

Salivary α-amylase was compared between the control (100), physically disabled (50) and mentally disabled (50) population. The normal range of salivary α-amylase level ranged from 1 to 371 U/ML on a wider range. A statistically significant value of salivary α-amylase was observed between the control and disabled groups (*P* = 0.000). The physically disabled group showed the highest mean and standard deviation (482.34 ± 56.52) compared with the mentally disabled (42.61 ± 33.87) and control (273.07 ± 73.33) groups. Salivary α-amylase was the least in the mentally disabled group [Figure 1a].

The comparison of salivary α-amylase based on age-wise distribution of study participants showed significant results. The participants less than 12 years of age were observed in the control (10) and physically disabled (2) groups but not in the mentally disabled group. The salivary α-amylase in the control (193 ± 61.19) and physically disabled (402.50 ± 6.36) groups presented a statistically significant difference (*P* = 0.001). Participants in 13–19 years (teenager group) also presented a significant difference between the control (242.76 ± 42.11) and physically disabled (mean 459.00) groups. There were no participants in the mentally disabled group between 13 and 19 years

of age group. The majority of participants were observed in more than 20 years of age group with control (69%), physically disabled (94%) and mentally disabled (100%) participants. The salivary α-amylase was significantly higher in the physically disabled group, followed by the control and mentally disabled groups (*P* = 0.000) [Figure 1b].

A gender-wise comparative analysis of salivary α-amylase presented a significant difference among groups (*P* = 0.000). In controls, salivary α-amylase levels in males (297.18 ± 68.56) were more compared with females (248.96 ± 70.57), whereas, in the physically disabled group, the salivary α-amylase levels in females (515.88 ± 43.59) were more compared with males (451.38 ± 49.39). A similar increase in salivary α-amylase levels in females (45.37 ± 39.61) than in males (41,06 ± 30.77) was observed in mentally disabled participants [Figure 1c].

DISCUSSION

The coronavirus disease 2019 (COVID-19) pandemic was the major cause of stress in the general population worldwide. Stress was measured using human body fluids. Human saliva is the most researched essential body fluid and has established its pivotal role as a safe and reliable indicator of stress. Salivary α-amylase is the most abundant protein, comprising 10 to 20% of the total protein content.[1] Numerous studies have stated that salivary α-amylase has a direct correspondence with the psychological stress.[1,6,9,10] Considering salivary α-amylase as a potent stress biomarker, the present study evaluated its level in control and disabled individuals, who suffered due to several factors in the pandemic. The scales of disability were defined by the international classification of functioning, which was followed in our study to differentiate between healthy and disabled individuals.[2]
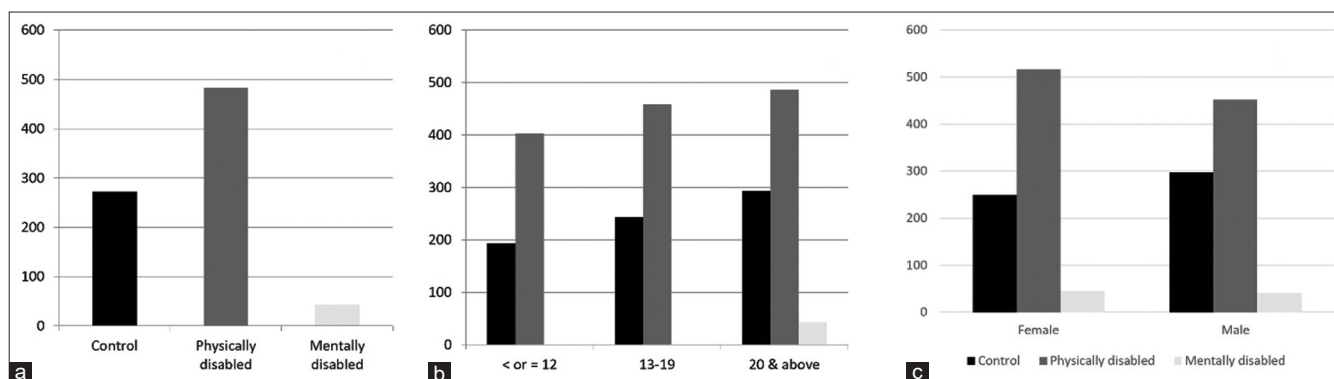

Figure 1: Salivary α-amylase levels among the groups are presented [Figure 1a-c]. The mentally disabled group presented the least level of salivary α-amylase compared with control and physically disabled groups [Figure 1a]. The levels of salivary α-amylase were highest in the physically disabled group with participants more than 20 years of age group compared with teenagers and less than 12 years of age group [Figure 1b]. Females presented higher salivary α-amylase levels in the physically disabled group compared with the mentally disabled group, whereas controls presented increased level of salivary α-amylase in males compared with females [Figure 1c].

Salivary α-amylase can be altered due to various environmental factors. It was reported that the activation of the noradrenergic pathways alters salivary α-amylase levels.[11] Salivary α-amylase levels drop after awakening and increase during the daytime.[11] Occupational stress and lower efficacy at the workplace are responsible for the higher levels of salivary α-amylase during the day but lower in the night.[12] Hence, to avoid such differences, we collected the salivary samples between 8 and 10 a.m. in a relaxed atmosphere to provide accurate values of salivary α-amylase levels both in the control and disabled population.

A study by Fischer *et al.* was conducted where salivary α-amylase levels in response to stress were studied in Turkish immigrants where negative remarks about their ethnicity had led to the increased reactivity of salivary α-amylase. This indicated a direct correlation between salivary α-amylase levels and stress.[13] In the present study, salivary α-amylase levels were high in the physically disabled group compared with the control and mentally disabled groups. This indicates an increase in the stress levels in the physically disabled group due to difficulty in performing daily tasks independently and inability to maintain social distancing, joblessness and financial instability, leading to increased stress level in the pandemic.

The salivary α-amylase levels were raised in the age group of 20 years and above as compared to the other two age groups, suggesting that the adult population was severely affected by stress during the pandemic. This could be due to restriction of mobility, financial stress and risk of disease contraction. The increase in salivary α-amylase in autistic children was presented earlier,[12,14] which contrasts with the present study where the physically disabled group presented higher salivary α-amylase levels compared with control and mentally disabled groups.

The level of stress could be different among males and females, and hence, salivary α-amylase could also show alterations. To our knowledge, we are the first to conduct and report a gender-wise comparison of salivary α-amylase and assess the stress levels in males and females in our study groups. We observed an increase in salivary α-amylase levels in the female population of physically disabled compared with males, indicating that females are more stressed and affected by the pandemic. The reason could be the limited accessibility and lack of interaction due to the strict COVID-19 protocols. However, in the control group the salivary α-amylase levels were slightly high in the males, signifying that the pandemic even had a deleterious effect on daily lives.

To conclude, this preliminary study highlighted the salivary α-amylase as a potent biomarker of stress. To conduct additional analysis of salivary biomarkers of stress, further studies are encouraged to develop the assay profiles and to define and distinguish between acute and chronic stress, anxiety, and depression. Saliva-based diagnostics have made the fluid important to reveal the connection of salivary changes in all aspects to systemic health status.

Financial support and sponsorship

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Arhakis A, Karagiannis V, Kalfas S. Salivary alpha-amylase activity and salivary flow rate in young adults. Open Dent J 2013;7:7-15.
2. World Health Organization. International Classification of Functioning, Disability, and Health: Children and Youth Version: ICF-CY. World Health Organization; 2007.
3. Ueda R, Okada T, Kita Y, Ozawa Y, Inoue H, Shioda M, *et al.* The quality of life of children with neurodevelopmental disorders and their parents during the coronavirus disease 19 emergency in Japan. Sci Rep 2021;11:3042.
4. Forness SR, MacMillan DL. Mental retardation and the special education system. Psychiatric Annals 1989;19:190-6.
5. Necho M, Birkie M, Gelaye H, Beyene A, Belete A, Tsehay M. Depression, anxiety symptoms, Insomnia, and coping during the COVID-19 pandemic period among individuals living with disabilities in Ethiopia, 2020. PLoS One 2020;15:e0244550.
6. Chojnowska S, Ptaszyńska-Sarosiek I, Kępka A, Knaś M, Waszkiewicz N. Salivary biomarkers of stress, anxiety and depression. J Clin Med 2021;10:517.
7. Chaturvedi Y, Chaturvedy S, Marwah N, Chaturvedi S, Agarwal S, Agarwal N. Salivary cortisol and alpha-amylase-biomarkers of stress in children undergoing extraction: An *in vivo* Study. Int J Clin Pediatr Dent 2018;11:214-8.
8. Santos SVMD, Silva LAD, Terra FS, Souza AV, Espindola FS, Marziale MHP, *et al.* Association of salivary alpha-amylase with anxiety and stress in nursing professionals. Rev Lat Am Enfermagem 2021;29:e3468.
9. Ali N, Nater UM. Salivary alpha-amylase as a biomarker of stress in behavioral medicine. Int J Behav Med 2020;27:337-42.
10. Vineetha R, Pai KM, Vengal M, Gopalakrishna K, Narayanakurup D. Usefulness of salivary alpha amylase as a biomarker of chronic stress and stress related oral mucosal changes-A pilot study. J Clin Exp Dent 2014;6:e132-7.
11. Rohleder N, Nater UM. Determinants of salivary alpha-amylase in humans and methodological considerations. Psychoneuroendocrinology 2009;34:469-85.
12. Kheur S, Deshpande R, Mahajan P, Bagde K, Dungarwal P, Rajpurohit LS, *et al.* A comparative evaluation of alpha amylase levels in autistic and normal children: A pilot study. Research Journal of Pharmaceutical, Biological and Chemicals sciences. January 2016;7:1510-4.
13. Fischer S, Nater UM, Strahler J, Skoluda N, Dieterich L, Oezcan O, *et al.* Psychobiological impact of ethnic discrimination in Turkish immigrants living in Germany. Stress 2017;20:167-74.
14. Danckaerts M, Sonuga-Barke EJ, Banaschewski T, Buitelaar J, Döpfner M, Hollis C, *et al.* The quality of life of children with attention deficit/hyperactivity disorder: A systematic review. Eur Child Adolesc Psychiatry 2010;19:83-105.