Original Article

Evaluation of Psychological Stress Marker in Partially Edentulous Indian Adults Restored with Fixed Dental Prosthesis – A Prospective Cohort Study

Srividyadevi Rajagopal, Naveen Gopi Chander, Kuttae Viswanathan Anitha, Muthukumar Balasubramaniam

Department of Prosthodontics, SRM Dental College, Chennai, Tamil Nadu, India

Abstract

Background: Clinical studies have established mastication as a stress relaxation behavior in humans. Absence of teeth compromises mastication, increasing psychologic stress in individuals depicted by many physiologic changes in body. Quantitative level of psychologic stress bio-markers serve as indicators of underlying physical ailment. Lesser literatures are available in determining the role of alpha amylase stress bio marker in partially edentulous clinical situations. **Aim:** The purpose of this clinical study was to evaluate the levels of salivary alpha-amylase (sAA) stress biomarker in partially edentulous subjects before and after restoration with fixed dental prosthesis. **Material and Methods:** Forty partially edentulous patients with missing mandibular first molar were selected for this study. Two questionnaires, state trait anxiety inventory (STAI) and perceived stress scale (PSS) was used to evaluate stress and anxiety levels of participants. The recruited participants were treated with metal ceramic fixed dental prosthesis (FDP). A visual analog scale (VAS) was used to determine the patient satisfaction .Unstimulated salivary samples were collected preoperative, 3rd and 6th month post FDP placement. Level of sAA was estimated. Data obtained in the form of mean ± SD was subjected to statistical analysis using paired sample t-test (α=.05). **Results:** The salivary alpha amylase level was highest with mean of 36.73 µM/min/mg ptn before restoration with FDP. In the third month after prosthesis placement, the enzyme values decreased to16.62 µM/min/mg ptn and least value of 8.58 µM/min/mg ptn was detected in sixth month ($P < 0.05$). **Conclusion:** The salivary alpha amylase stress biomarker decreased after tooth replacement with FDP.

Keywords: *Alpha-amylase, biomarkers, dental prosthesis, saliva*

Introduction

Loss of teeth leads to functional or structural disturbances to hard and soft oral tissues causing resorption of bone, supra-eruption of opposing teeth, shifting of teeth towards edentulous space, malocclusion leading to TMJ problem, loss of facial aesthetics, depreciation in nutrition, difficulty in speech and mastication; hence, replacement of lost teeth is mandatory to improve overall physical, mental and social health of patients.[1,2] Various options are available for the replacement of missing teeth, which include removable partial dentures, fixed dental prostheses (FDP), complete denture , and implants.[3] FDP is the widely used restoration for the replacement of missing teeth.

Psychologic stress can raise due to dental, systemic, and social disorders.[4,5] Studies have proven the association between mastication and parafunctional activities such as nail-biting, lip biting, or cheek biting as stress adaptation behavior.[6] The acquired habits can alter the levels of stress markers due to the changes arising in the hippocampus and hypothalamus.[7]

The psychological stress is evaluated through various markers present in the blood, urine, saliva, cerebrospinal fluid, gingival crevicular fluid.[8] The saliva is a noninvasive Marker that requires no special equipment for collection; storage is easier when compared to other fluids. Numerous salivary biomarkers were evaluated in the literature. Salivary alpha-amylase (sAA) has a direct correlation and better association between neuronal activities of sympathetic and parasympathetic branches of the autonomic nervous system (ANS).[9] Several questionnaires such as perceived-stress-scale (PSS), State-Trait-Anxiety Inventory-state scale,

How to cite this article: Rajagopal S, Chander NG, Anitha KV, Balasubramaniam M. Evaluation of psychological stress marker in partially edentulous Indian adults restored with fixed dental prosthesis – A prospective cohort study. Contemp Clin Dent 2020;11:116-20.

Submitted : 23-Jan-2020
Revised : 17-May-2020
Accepted : 27-May-2020
Published : 07-Aug-2020

Address for correspondence:
Dr. Kuttae Viswanathan Anitha, 76/110 Flat F-1 Chakra Enclave, Bajanai Koil Street, Choolaimedu, Chennai - 600 094, Tamil Nadu, India.
E-mail: dranikv@yahoo.co.uk

Access this article online

Website: www.contempclindent.org

DOI: 10.4103/ccd.ccd_63_20

Quick Response Code:

Ardell Wellness Stress Test, visual analog scale, and social readjustment rating scale are available to measure the stress, anxiety, and depression syndromes.[10-12]

Numerous studies are done to determine the levels of sAA as an indicator for diagnostic and therapeutic intervention for psychological stress.[13,14] In prosthodontic research, the authors are unaware of studies that have evidenced the replacement of missing teeth with fixed restorations reduced psychologic stress of patients with the help of biomarkers. Therefore, the purpose of the study was to identify the levels of sAA in patients restored with fixed dental prosthesis before and after treatment.

Methods

A prospective cohort study was carried out to investigate the levels of sAA stress biomarker level in forty partially edentulous subjects. Partially edentulous patients with missing mandibular first molar were selected and those who gave consent to participate in the study were included. They were Indian adults who reported to the department of prosthodontics at SRM dental college, Ramapuram, Chennai, India. Patient selection was made based on definitive inclusion and exclusion criteria. The partial edentulism index of "American College of Prosthodontists" was used for standardization of study. Class I category of patients of Prosthodontic Diagnostic Index were involved.[15] They represented an uncomplicated clinical scenario, with one missing tooth confined to a lower posterior quadrant, did not warrant preprosthetic procedures, abutment teeth were physiologically sound, Class I molar relationship existed, residual ridge structure corresponded to Class I complete edentulism characterization.

Both male and female subjects from 20 to 40 years of age, with missing mandibular first molar, were selected for the study. Stable physical and psychological health, first-time denture wearers with the partial edentulous period of maximum 6 months, class I molar relationship, devoid of spacing, misalignment, and temporomandibular joint disorder were used as inclusion criteria in the study. Subjects with psychotic disorders, systemic illness, those under lifetime medications, pregnancy, smokers, those under steroid therapy in last three months, autoimmune diseases, multiple missing teeth, previous denture wearers, completely edentulous state, salivary gland disorders, obesity, renal disease, those suffering from eating disorders such as anorexia nervosa and bulimia nervosa, were all excluded from the study.[15]

The selected subjects were requested to fill the State Trait Anxiety Inventory (STAI) and PSS questionnaire (Sheldon Cohen) to analyze the anxiety and psychological stress levels of participants. Participants with low stress and anxiety level scores as per the questionnaire were selected for the study. Missing tooth of recruited participants was rehabilitated with conventional full veneer metal-ceramic FDP. Standardized treatment protocol for fixed dental prosthesis was followed.

Salivary samples were collected before the intervention, 3rd, and 6th months post intervention. A standard method for the collection of salivary samples was followed.[16] Appointments were scheduled in the morning between 10 AM and 2 PM at least 45 min after brushing teeth. Participants were refrained from the intake of any food or beverages, performing laborious physical activity before the appointment. No dental therapy was performed 24 h before sample collection. They were also restricted from consumption of alcohol, caffeine, nicotine, or any forms of drugs 12 h prior to appointment. On the day of sample collection, they were instructed to rinse their mouth with mouth wash to expel food residues, if any. After a waiting period of 15 min, salivary samples were collected. Unstimulated saliva from the participant was acquired using a passive drool method in a sterile plastic 5 mL cryovial (Chenchems Pvt Ltd, India). The patient had to pool saliva under the tongue and had to gently propel the saliva into the collection unit by bending their head downward. The collected sample was centrifuged (Remi R-8C Laboratory centrifuge, India) at 3000 ×*g*, 10 min to obtain a supernatant solution, which was eventually shifted to another cryovial using a pipette and stored at -80ºc. An insulator freezer box (Gelid Thermocol box, India) was used for transportation of samples to testing laboratory (Herbal Indian Medicine and Research Laboratory, Sri Ramachandra University, India) for the estimation of sAA.[17]

The levels of alpha-amylase for all the time intervals were documented for all the forty subjects. A paired-sample *t*-test was done to compare mean values between different intervals. An independent-sample *t*-test was performed to evaluate the mean values between the sexes. The data were analyzed using (SPSS v16; SPSS Inc), Chicago, IL, USA.

Results

Initially, 57 patients fulfilled the prosthodontic criteria for the need of a fixed dental prosthesis. Later, when they filled the stress analysis questionnaire, 17 of the subjects became ineligible because of high STAI and PSS scores. Finally, forty subjects befitted the eligibility criteria. Of

Table 1: Paired-sample *t*-test to compare salivary alpha amylase mean values between different time intervals

Pair	Variable	*n*	Mean±SD	*P*
Pair 1	Preoperative, 3rd month (μM/min/mg ptn)	40	36.73±0.754	0.000
Pair 2	Preoperative, 6th month (μM/min/mg ptn)	40	16.62±0.856	0.000
Pair 3	Third month, 6th month (μM/min/mg ptn)	40	8.58±0.592	0.000

SD: Standard deviation

forty, 22 were men and 18 were women with a mean age of 30.98 ± 28 years for males and 30.83 ± 13 years for females, respectively.

The overall mean values of sAA in all forty individuals before placement of fixed dental prosthesis was 36.73 μM/min/mg ptn. A mean value of 16.62 μM/min/mg ptn and 8.58 μM/min/mg ptn was estimated during the 3rd and 6th month postoperatively. The highest levels of sAA levels were found before tooth restoration and over the periodic evaluation, the values decreased with least found at the end of the 6th month postoperatively. The paired-sample *t*-test revealed statistically significant difference was obtained between the mean values of preoperative and postoperative enzyme levels ($P < 0.05$), as shown in [Table 1]. On the comparison between genders, a statistically insignificant difference ($P > 0.05$) between both sexes was established [Table 2]. Negligible numerical variation in sAA levels between them was detected. In males, preoperatively, it was 36.55 μM/min/mg ptn, 16.23 μM/min/mg ptn, and 8.58 μM/min/mg ptn were at the end of 3rd and 6th month after tooth restoration. In females it was 30.13 μM/min/mg ptn, 15.88 μM/min/mg ptn, and 7.95 μM/min/mg ptn along the study periods.

Discussion

sAA which is secreted on the adrenergic activation process, is proven to be an objective measure to identify stress levels in subjects. Physiological response to stress activates both central and peripheral nervous systems. In the central nervous system, the hypothalamus and brainstem are stimulated. While the peripheral nervous system involves outflow from the hypothalamic-pituitary-adrenal axis and the ANS– which constitutes the efferent sympathetic-adrenal-medullary system and parasympathetic system. sAA is synthesized by the main secretory acinar cells of salivary glands. Neuronal activation by stress causes the release of the enzyme stored in granules before secretion into saliva. Proctor and carpenter[18] stated that the amount of amylase that is secreted per unit of time is directly proportional to the extent of sympathetic activity and was a better representation of neuronal activity. In this study, amylase output per unit time was calculated to directly reflect the same. Granger *et al*.[19] determined that the association of stress, level of sAA, and variations in the body. Its evaluation in the saliva is an established, simple, noninvasive method for investigation of psychotic stress in individuals undergoing treatment. Schumacher *et al*.[13] observed the peculiar molecular structure of salivary amylase, making it unique when compared to other biomarkers and making it one of the most commonly used markers in bio-behavioral research. Asking *et al*.,[20] Speirs *et al*.[21] Chatterton *et al*.,[22] Soeda *et al*.,[23] Ishiyama *et al*.,[24] Skosnik *et al*.,[25] Takai *et al*.[26] observed an increased level of sAA activity when ANS combines both sympathetic and parasympathetic activation of the salivary gland.

Table 2: Independent sample *t*-test to compare salivary alpha amylase mean values between sexes

Variable	Sex	*n*	Mean±SD	*P*
Age	Male	22	30.28±2.87	0.392
	Female	18	30.13±3.14	
Preoperative (μM/min/mg ptn)	Male	22	36.55±1.22	0.548
	Female	18	36.95±1.38	
3rd month (μM/min/mg ptn)	Male	22	16.23±0.602	0.885
	Female	18	15.88±0.775	
6th month (μM/min/mg ptn)	Male	22	8.58±0.317	0.677
	Female	18	7.95±0.421	

SD: Standard deviation

The definitive association between missing teeth, stress, sAA levels, and restoration were used as research questions in the current study. This study was hypothesized to identify the changes of salivary sAA with the restoration of lost missing teeth. The levels of sAA was estimated before restoration with conventional FDP and after 3rd and 6th month of restoration. The study found that a statistically significant difference was found in the pre-operative and postoperative mean sAA levels in all forty subjects. Increased sAA levels were found before the placement of FDP, and the decreased level was found in the post follow-up after the placement of the prosthesis. The results are in correspondence in research done by Chatterton *et al*.,[22] Gordis *et al*.[19] and Nater *et al*.[27] who observed elevated sAA enzyme levels in many stress contributing actions such as sports activities, mental aptitude tests. Here in this study absence of tooth contributed to stress escalation of sAA levels. The previous studies evidenced that sAA levels altered due to nor-adrenergic response. Breseghelo *et al*.,[28] Bosch *et al*.,[29] Sadi *et al*.,[30] Niwa *et al*.[31] determined the reduction in sAA during the mastication process due to the activity of the sympathetic nervous system. The mastication increased activity of the prefrontal cortex leading to a reduction in stress markers by the release of histamines H1 from hippocampus and activation of the H1 receptor. These are the scientific correlations from previous literature to support that reduction in sAA biomarker levels resulted in a decrease in the stress of any origin.

In the current study, insignificant difference was found between both males and females during all three intervals of sAA estimation. Contradictory results were observed by van Stegeren *et al*.[32] where men had higher baseline values of sAA than females in un-stimulated salivary samples. But Filaire *et al*.[33] and Nater *et al*.[26] found that there was no gender difference in sAA levels. In contrast, Rohleder *et al*.[34] observed levels of sAA influenced by cigarette smoking, in which he found higher levels of sAA in women than men.

There are several direct, indirect, or mediator response-related factors causing stress to the patients. There

is no direct cause and effect relationship derived at the end of the study. Negligence to the restoration of even a single back tooth can predispose the individual to physiological elevation in stress biomarker levels, as inferred from the rehabilitation of molar tooth in a clinical situation. Utmost care was taken in the recruitment of cohorts for the study. Preliminary elimination of subjects with the help of stress and anxiety questionnaires enabled to control variables before rehabilitation.

Control of confounding factors was done by stringent standardization protocol from the recruitment of the most eligible cohorts and sound operational practice during the conduct of the study. Periodic monitoring of the biomarker levels was done at different stages to rule out the coincidental relationship between the two variables. Nevertheless, more prospective clinical trials are needed to find out the direct cause and effect association between tooth rehabilitation and sAA biomarker.

The observations made was subjected to the participant and situational variables during the study. A statistical significant association between levels of salivary alpha amylase and restoration of teeth with FDP does not directly imply the causation of psychological stress in participants. With control of variables, the rehabilitation of missing teeth with FDP restoration acts as a variable component reducing the levels of stress biomarkers. It was proved that increases in amylase activity may be one of many actions involved in activating the body's resources to cope with the stressful event such as the loss of tooth to masticate food. sAA was studied as an acute stressor element. Further studies are needed to examine chronic changes in sAA concentrations. If the clinical condition of long-term edentulism causes sustained elevation of sAA levels due to ANS activity, then it can be detrimental to both the physiological and psychological health of the patient.[35]

Till date, in evidence-based prosthodontic research, only limited studies on salivary stress bio-markers are done. As a future scope, further studies can incorporate the investigation of other salivary biomarkers apart from sAA to identify their significance in edentulous patients. Different types of dental prostheses and their correlation with biomarkers can be studied prospectively. For the physical and psychological well-being of edentulous patients, more such prospective clinical trials are required to increase immediate awareness in the replacement of missing teeth. The following conclusions were obtained within the limitations of the study.

1. The preoperative mean values before cementation of FDP was 36.73 μM/min/mg ptn
2. Postoperative mean values of sAA after 3 months of placement of FDP were 16.62 μM/min/mg ptn. Mean values after 6 months of placement of FDP was 8.58 μM/min/mg ptn
3. sAA stress biomarker decreased with FDP tooth replacement.

Clinical implications

With respect to clinical relevance, it is to draw evidence that immediate rehabilitation of missing teeth is of paramount importance to psychotic health. Any delay in attention can deteriorate the well-being of subjects as reflected by levels of stress biomarkers. This biological indicator levels predict the development of stress-related pathologies and behavioral changes in edentulous subjects. It is well agreed that sAA is a surrogate marker of central nervous system activity, and its levels are a representative outcome. The observations made were subjected to the participant and situational variables during the study. The statistical significant association between levels of sAA and restoration of teeth with FDP does not directly imply the causation of psychological stress in participants. With control of variables, the rehabilitation of missing teeth with fixed dental prosthesis restoration acts as a variable component reducing the levels of stress biomarker.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Soboļeva U, Lauriņa L, Slaidiņa A. The masticatory system – An overview. Stomatologija 2005;7:77-80.
2. Gerritsen AE, Allen PF, Witter DJ, Bronkhorst EM, Creugers NH. Tooth loss and oral health-related quality of life: A systematic review and meta-analysis. Health Qual Life Outcomes 2010;8:126.
3. Pjetursson BE, Lang NP. Prosthetic treatment planning on the basis of scientific evidence. J Oral Rehabil 2008;35 Suppl 1:72-9.
4. Zarb GA. The replacement of missing teeth. Can Fam Physician 1988;34:1435-40.
5. Cohen S, Janicki-Deverts D, Miller GE. Psychological stress and disease. JAMA 2007;298:1685-7.
6. Slavicek R, Sato S. Bruxism – A function of the masticatory organ to cope with stress. Wien Med Wochenschr 2004;154:584-9.
7. Ono Y, Yamamoto T, Kubo KY, Onozuka M. Occlusion and brain function: Mastication as a prevention of cognitive dysfunction. J Oral Rehabil 2010;37:624-40.
8. Ma L, Wan J, Shen X. Salivary alpha-amylase and behavior reaction in acute stress and the impact of tridimensional personality. Adv Exp Med Biol 2018;1072:431-6.
9. Petrakova L, Doering BK, Vits S, Engler H, Rief W, Schedlowski M, *et al*. Psychosocial stress increases salivary alpha-amylase activity independently from plasma noradrenaline levels. PLoS One 2015;10:e0134561.
10. Ezzati A, Jiang J, Katz MJ, Sliwinski MJ, Zimmerman ME, Lipton RB. Validation of the perceived stress scale in a community sample of older adults. Int J Geriatr Psychiatry 2014;29:645-52.
11. Cai W, Dong W, Pan Y, Wei C, Zhang S, Tian B, *et al*. Reliability, validation and norms of the Chinese version of

anxiety sensitivity index 3 in a sample of military personnel. PloS One 2018;13:e0201778.
12. Brokelman RB, Haverkamp D, van Loon C, Hol A, van Kampen A, Veth R. The validation of the visual analogue scale for patient satisfaction after total hip arthroplasty. Eur Orthop Traumatol 2012;3:101-5.
13. Schumacher S, Kirschbaum C, Fydrich T, Ströhle A. Is salivary alpha-amylase an indicator of autonomic nervous system dysregulations in mental disorders? – A review of preliminary findings and the interactions with cortisol. Psychoneuroendocrinology 2013;38:729-43.
14. Klaus K, Doerr JM, Strahler J, Skoluda N, Linnemann A, Nater UM. Poor night's sleep predicts following day's salivary alpha-amylase under high but not low stress. Psychoneuroendocrinology 2018;101:80-6.
15. McGarry TJ, Nimmo A, Skiba JF, Ahlstrom RH, Smith CR, Koumjian JH, *et al*. Classification system for partial edentulism. J Prosthodont 2002;11:181-93.
16. Rashkova MR, Ribagin LS, Toneva NG. Correlation between salivary alpha-amylase and stress-related anxiety. Folia Med (Plovdiv) 2012;54:46-51.
17. Bernfeld P. Amylases α and β. In: Clowick SP, Kaplan NO. Methods in Enzymology. Vol. 1. New York: Academic Press Inc Publishers; 1955. p. 149-52.
18. Proctor GB, Carpenter GH. Regulation of salivary gland function by autonomic nerves. Auton Neurosci. 2007;133:3-18.
19. Gordis EB, Granger DA, Susman EJ, Trickett PK. Asymmetry between salivary cortisol and alpha-amylase reactivity to stress: Relation to aggressive behavior in adolescents. Psychoneuroendocrinology 2006;31:976-87.
20. Asking B. Sympathetic stimulation of amylase secretion during a parasympathetic background activity in the rat parotid gland. Acta Physiol Scand 1985;124:535-42.
21. Speirs RL, Herring J, Cooper WD, Hardy CC, Hind CR. The influence of sympathetic activity and isoprenaline on the secretion of amylase from the human parotid gland. Arch Oral Biol 1974;19:747-52.
22. Chatterton RT Jr., Vogelsong KM, Lu YC, Hudgens GA. Hormonal responses to psychological stress in men preparing for skydiving. J Clin Endocrinol Metab 1997;82:2503-9.
23. Soeda R, Tasaka A, Sakurai K. Influence of chewing force on salivary stress markers as indicator of mental stress. J Oral Rehabil 2012;39:261-9.
24. Ishiyama I, Suzuki M, Sato M, Nakamura T. Application of heart rate variability, salivary constituents and electroencephalographic elements to evaluate function of the sympathetic or parasympathetic nerves during masticatory exercise. J Masticat Health Soc 2006;16:55-69.
25. Skosnik PD, Chatterton RT Jr., Swisher T, Park S. Modulation of attentional inhibition by norepinephrine and cortisol after psychological stress. Int J Psychophysiol 2000;36:59-68.
26. Takai N, Yamaguchi M, Aragaki T, Eto K, Uchihashi K, Nishikawa Y. Effect of psychological stress on the salivary cortisol amd amylase levels in the healthy young adults. Arch Oral Biol 2004;49:963-8.
27. Nater UM, La Marca R, Florin L, Moses A, Langhans W, Koller MM, *et al*. Stress-induced changes in human salivary alpha-amylase activity – Associations with adrenergic activity. Psychoneuroendocrinology 2006;31:49-58.
28. Breseghelo Mde L, Guillo LA, Nogueira TE, Leles CR. Nitric oxide concentration and other salivary changes after insertion of new complete dentures in edentulous subjects. Int J Dent 2016;2016:8351427.
29. Bosch JA, de Geus EJ, Veerman EC, Hoogstraten J, Amerongen AV. Innate secretory immunity in response to laboratory stressors that evoke distinct patterns of cardiac autonomic activity. Psychosom Med 2003;65:245-58.
30. Sadi H, Finkelman M, Rosenberg M. Salivary cortisol, salivary alpha amylase, and the dental anxiety scale. Anesth Prog 2013;60:46-53.
31. Niwa M, Hiramatsu I, Nakata F, Hamaya C, Onogi N, Saito K. Functional significance of stress-relieving act of chewing and it effect on brain activation by stress. Nihon Nouson Igakukai Zasshi 2005;54:661-6.
32. van Stegeren A, Rohleder N, Everaerd W, Wolf OT. Salivary alpha amylase as marker for adrenergic activity during stress: Effect of betablockade. Psychoneuroendocrinology 2006;31:137-41.
33. Filaire E, Dreux B, Massart A, Nourrit B, Rama LM, Teixeira A. Salivary alpha-amylase, cortisol and chromogranin A responses to a lecture: Impact of sex. Eur J Appl Physiol 2009;106:71-7.
34. Rohleder N, Wolf JM, Maldonado EF, Kirschbaum C. The psychosocial stress-induced increase in salivary alpha-amylase is independent of saliva flow rate. Psychophysiology 2006;43:645-52.
35. Sarnyai Z, Berger M, Jawan I. Allostatic load mediates the impact of stress and trauma on physical and mental health in Indigenous Australians. Australas Psychiatry 2016;24:72-5.