Journal of Neonatal Surgery
ISSN(Online): 2226-0439
Vol. 13, (2024)
https://www.jneonatalsurg.com

OPEN ACCESS

Assessment of Salivary stress marker in Completely Edentulous Patients and Its association with Residual Ridge Resorption: A cross sectional study

Divyansh Sinha [1], Subhabrata Maiti[2], Selvaraj J[3], Jessy P[4], Allour Vaishnavi[5],

[1]Post Graduate , Department of Prosthodontics, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai-77,Tamil Nadu,India.

[2]Associated Professor ,Department of Prosthodontics,Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University,Chennai-77,Tamil Nadu,India.

Email ID: drsubhoprostho@gmail.com

[3]Professor,Department of Pharmacology, Saveetha Dental College & Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University,Chennai-77.

[4]Assistant Professor , Department of Pedodontics, Saveetha Dental College and Hospitals,Saveetha Institute of Medical and Technical Sciences,Saveetha University,Chennai-77,Tamil Nadu,India.

Email ID : drjessy2019@gmail.com

[5]Department of Prosthodontics,Saveetha Dental College and Hospitals, Saveetha Institute of Medical And Technical Sciences,Saveetha University,Chennai-77,Tamil Nadu,India.

Email ID: 151801005.sdc@saveetha.com

Corresponding Author:

Subhabrata Maiti,

Associated Professor,Department of Prosthodontics,Saveetha Dental College and Hospitals,Saveetha Institute of Medical and Technical Sciences, Saveetha University,Chennai-77,Tamil Nadu,India.

Email ID: drsubhoprostho@gmail.com

Cite this paper as: Divyansh Sinha , Subhabrata Maiti, Selvaraj J, Jessy P, Allour Vaishnavi, (2024) Assessment of Salivary stress marker in Completely Edentulous Patients and Its association with Residual Ridge Resorption: A cross sectional study. *Journal of Neonatal Surgery*, 13, 1633-1640.

ABSTRACT

AIM & BACKGROUND: Residual ridge resorption (RRR) is an ongoing inflammatory process that can result in tissue damage, particularly through the formation of free radicals, which can lead to bone resorption via various pathways and mechanisms. The aim of this study was to assess and explore the connection between cortisol levels, a stress marker found in saliva, and the extent of residual ridge resorption in individuals who are completely edentulous, with or without dentures.

METHODS: In this research, a total of 60 participants between the ages of 45 and 70, who had no natural teeth, were included. Saliva samples were gathered in sterile containers. The mean levels of cortisol were evaluated using ELISA kit and radiographic examination of remaining bone level at the mental foramen region was assessed. To analyze the data, we employed an independent t-test to compare two groups: those who wore dentures and those who did not. Additionally, we conducted Pearson and Spearman correlation analyses within the SPSS software to evaluate the potential relationship between cortisol levels and bone loss.

RESULTS: The findings indicated a notable reduction in cortisol levels, a salivary stress marker, among patients who wore dentures in comparison to those who did not wear dentures (p=0.001).The strong negative correlation (-0.81 & -0.94) was found between cortisol and residual bone.

CONCLUSION: Cortisol levels exhibited a notable reduction in individuals wearing dentures as opposed to those without dentures. Concurrently, long-term denture wearers experienced a decrease in residual ridge resorption, which suggests a decrease in stress markers and subsequently less pronounced residual ridge resorption.

CLINICAL SIGNIFICANCE : The use of dentures may contribute to reduced cortisol levels and less residual ridge resorption in long-term wearers, potentially indicating improved oral health and reduced stress-related oral changes leading to a great situation for implant too..

Keywords: *Cortisol, complete dentures, saliva, residual ridge resorption, innovation*

1. INTRODUCTION

In the realm of oral health, a profound challenge confronts completely edentulous patients: the inexorable process of residual ridge resorption.Completely edentulous patients, those who have lost all their natural teeth, often face the daunting prospect of residual ridge resorption. This natural process entails the gradual loss of alveolar bone structure, which once supported the teeth. As this ridge diminishes in size and quality, it can lead to difficulties in denture retention and overall oral health.While residual ridge resorption is a well-documented phenomenon, its underlying causes and contributing factors remain subjects of extensive study.[1] Emerging research has increasingly highlighted the role of stress as a potential catalyst for various health issues, including those affecting the oral cavity.While there is an increasing focus on examining the effects of stress on overall well-being, there is still a significant lack of knowledge regarding how psychological stress factors might affect oral health, particularly in relation to residual ridge resorption.

An entity characterized by having one or more unpaired electrons, capable of existing independently, is termed a free radical (FR). Free radicals use multiple processes, including DNA oxidation, lipid peroxidation, protein oxidation, the disruption of vital enzymes, and the activation of pro-inflammatory cytokines, to cause damage to tissues. Given that residual ridge resorption (RRR) signifies a persistent inflammatory state, these generated free radicals have the potential to provoke tissue damage, especially in relation to bone resorption.[2-4] Lipid peroxidation stands as one of the potential contributing processes. The body's defense mechanisms respond to free radicals in three distinct ways: they can either eliminate, scavenge, or expel them. Oxidative stress arises when there is a disruption in the equilibrium between the body's protective antioxidant mechanisms and the generation of reactive oxygen species (ROS) or free radicals (FR). Indicators for the antioxidant defense system encompass both enzyme-based antioxidants like superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase, as well as non-enzyme-based antioxidants like uric acid, vitamin C, and vitamin E.The specific type of process leading to tissue damage determines the biomarkers associated with free radicals. These biomarkers enable us to assess the nature and severity of the damage resulting from the inflammatory event, providing valuable insights into the scope and advancement of the disease process.[5-7]

The enzyme superoxide dismutase plays a vital role in safeguarding cellular health. In humans, there exist three distinct SOD isoenzymes: Cu/Zn SOD, located in the cytoplasm and nucleus; Mn-SOD, found within the mitochondria; and EC-SOD, located outside the cells. Its primary function involves facilitating the conversion of O2 into H2O2, which effectively eliminates harmful reactive oxygen species (ROS) from the cellular environment. Oxidative stress, which leads to lipid peroxidation, is known to result in tissue damage, particularly in the context of bone resorption. A notable byproduct of lipid peroxidation is cortisol.The present study aims to examine cortisol levels in individuals who have lost all their teeth and establish connections between these cortisol levels, the types of bone resorption, the duration of edentulism, the influence of denture use, age, and gender. Given the limited existing research on the assessment of salivary stress markers, specifically cortisol, in completely edentulous patients, this experimental investigation seeks to evaluate cortisol levels in this particular group.

Present study carries significance not only in providing potential insights into the interplay between stress and oral health but also in advancing our knowledge of how to better support the oral well-being of completely edentulous patients.In this cross-sectional clinical investigation, the primary objective is to examine the existence and concentrations of salivary stress markers in individuals who are entirely without teeth and explore whether these markers have any connection with the development and advancement of residual ridge resorption.The null hypothesis of the study was grounded in the hypotheses that changed levels of salivary stress markers will not be observed in completely edentulous patients, and these markers will not play a statistically significant association with the severity of residual ridge resorption.

2. MATERIAL AND METHODS:

Study design

A cross-sectional Clinical study.

Sample size calculation:

This cross-sectional research was carried out within the hospital university environment at the Department of Prosthodontics in Chennai. The study focused on individuals aged 40 to 65 years who had lost all of their teeth. The sample size, consisting of 60 participants, was determined using G-Power software with a power of 80 and a significance level of 0.005. 30 samples for each group (denture wearer and non denture wearer) was taken based on inclusion and exclusion criteria **(Figure 1)**.

Ethical Approval:

Ethical clearance for the study was secured from the institutional ethics committee, under the reference number IHEC/SDC/UG-1805/22/PROSTHO/629. Only those individuals who willingly agreed to participate and provided their formal consent were included in the study.

Selection criteria

Inclusion

Completely edentulous denture wearing patients and patients who were not wearing any denture for 1 year were considered as inclusion criteria for control , and edentulous patients with denture immediate after extraction and wearing denture for 10 months to 1 year were selected as an experimental group.

Exclusion

Individuals with systemic illnesses, as well as those who had conditions such as gout and arthritis, along with individuals who were smokers or had a history of alcohol consumption, were not considered for inclusion in the study. Patients with long span edentulous areas for a long time prior to extraction were eliminated . edentulous area more than one year or less than six months was eliminated.

Measurement of Residual bone

A standardized form was completed for each patient, and an orthopantomogram (OPG) was obtained for each individual using the same machine and established procedure **(Figure 2)**. The form recorded the mandibular bone height at the mental foramen level, with measurements taken from the top surface of the foramen to the upper bone surface.

Collection of saliva

1 ml of saliva sample was taken in a microcentrifuge tube and was centrifuged at 4000 rpm for 10 mins at 4°C in a cooling centrifuge. The supernatant was transferred to a fresh microcentrifuge tube and it is stored at -20°C for further experimental use.

Quantification of Cortisol:

Human Cortisol ELISA kit employs a two-site sandwich ELISA to quantify cortisol levels in the clinical samples. A microplate was prepared in advance with an antibody that specifically targets cortisol. Subsequently, both standards and samples were processed concurrently and underwent an incubation period. Cortisol present in the sample were bound to the pre coated antibody and unbound substances were removed by washing. Then Horseradish Peroxidase (HRP) enzyme was added and incubated. After incubation washing step was carried out followed by the addition of chromogen substrate solution which was added & incubated at dark environment. The color developed is proportional to the amount of cortisol present in the sample. The intensity of color developed was measured at 450 nm in the ELISA plate reader.

Statistical Analysis

The data were initially entered and documented in Microsoft Excel 2016 (Microsoft Office 10), and subsequently, they were transferred to SPSS (Statistical Package for Social Science, Windows Version 20.0) by SPSS Inc., located in Chicago, USA. Following this, thorough statistical analyses were conducted. These analyses encompassed the application of an Independent T-test, where the significance level was set at $p<0.05$, and the calculated p-values were a result of this independent t-test. Furthermore, Pearson and Spearman correlation assessments were executed within the SPSS software to investigate potential associations between cortisol levels and the extent of bone loss.

3. RESULTS:

The results showed that cortisol levels were significantly decreased (p=0.001) in denture wearer patients (52.36±4.03) when compared to the patients without denture(63.03± 10.5) **(Table 1)**. Also there was a significant difference of bone height (p=0.001) of denture wearers were with non denture wearer (8.23± 10.5) individuals depicting the less residual ridge resorption in the patient with denture(14.21± 4.03) **(Table 2)**. There was strong negative correlation between stress marker cortisol level and residual bone **(Figure 3)**. Spearman correlation coefficient was -0.811 and Pearson correlation was -0.944 indicating strong negative correlation **(Table 3)**.

4. DISCUSSION:

The objective of this study was to assess salivary cortisol levels in individuals who have lost all their teeth. Our research unveiled a notable difference in mean values at the initial stage among the various groups, with a statistically significant result (p=0.001).Notably, the mean cortisol level in patients wearing dentures was notably lower than in patients without dentures, and this difference was statistically significant (p=0.001).Prior research findings have consistently shown that clinical studies have yielded favorable outcomes with dental implants in patients who use dentures. Moreover, these studies have demonstrated a reduction in ridge resorption in denture-wearing patients compared to those who have been without dentures for an extended period.[8] This study has unveiled a noteworthy finding, indicating that the mean value of residual ridge height in patients who wear dentures stands at 14.21±4.03mm, and this difference is statistically significant. In contrast, patients without dentures exhibit a mean height of 8.23±10.5mm. This discrepancy underscores that patients who do not use dentures experience more pronounced resorption of the residual ridge.Residual ridge resorption represents a phenomenon in

which the loss of teeth leads to a permanent reduction in the size of the ridges. Rehabilitating edentulous patients with substantial residual ridge resorption poses a considerable challenge. This is primarily due to the enduring catabolic remodeling of the remaining bone architecture, a process that is both unique and continuous. This phenomenon commences at the time of tooth extraction and persists throughout an individual's lifetime.[9] After the removal of a tooth, a series of inflammatory mediators is activated, and these elements subsequently play a role in the process of residual ridge resorption (RRR).The mandible, owing to its comparatively smaller surface area when compared to the maxilla, invariably experiences a discrepancy in the extent of resorption between the two. Kovacić and colleagues identified that after a year, RRR in the mandible is 2.5 times greater than in the maxilla.

Notably, both the upper (maxillary) and lower (mandibular) remaining ridges display a greater level of residual ridge resorption (RRR) at their frontal locations, as opposed to their lateral areas.It's worth noting that the pace and extent of resorption vary not only among individuals but also within the same individual over time and space. In this research, utilizing the biomarker cortisol, our objective was to investigate the link between oxidative stress and antioxidants concerning residual ridge resorption (RRR) in individuals with total tooth loss.[10] The production of ROS is increased when macromolecules suffer oxidative damage. ROS production is essential for the onset of inflammation and illness. Evaluating the by-products that are produced by this system is one way to study it. Cortisol, the primary and extensively studied element found in both saliva and gingival crevicular fluid (GCF), is recognized as a dependable indicator of bone damage caused by oxidative stress, resulting in bone resorption due to lipid peroxidation. Cortisol was validated as a biomarker of oxidative harm.[11–13] This research is related to increased occurrences of tooth loss and bone-related ailments, even though there haven't been any prior studies conducted on individuals without teeth (edentulous individuals). Related to periodontitis in patients with dentures study was done but with edentulous patient the data are limited. Early edentulism was associated with a larger increase in cortisol levels, which might be a sign of enhanced ridge resorption.Based on the research conducted by Carlsson and Persson, it was observed that during the initial year of edentulism, the rate of resorption increased at a pace of 0.13 mm per year before gradually decreasing to 0.5 mm per year. In accordance with the findings by Kovaci et al., individuals who had more recently lost their last remaining teeth experienced a more significant reduction in alveolar bone height.[14]

As per a range of extensive studies, well-fitted dentures serve as a safeguard for the alveolar ridge. When promptly applied following tooth extraction, dentures have the potential to contribute to the preservation of the buccal plate of the bone. The current body of literature regarding this subject suggests that the utilization of immediate dentures can potentially mitigate the extent of residual ridge resorption (RRR). The use of dentures after extraction appears to be correlated with increasing permanent bone loss. Studies conducted by Bergman et al.[15] did not find any statistically substantial association between the use of dentures and the pace of residual ridge resorption (RRR). According to research by Barbo, wearing dentures frequently raises RRR. Again evidence shows the positive views towards denture and additionally denture with soft liner.[16–18] In previous research study their findings suggested that wearing dentures can be protective for the underlying mucosa.[19,20] The protective impact is evident because both denture wearers and non-denture wearers displayed indications of inflammatory cell infiltration, but individuals who did not wear dentures had a more pronounced concentration of inflammatory cells. Our research findings are consistent with this observation, as they demonstrated that those not wearing dentures had higher cortisol levels, with a statistically notable disparity in cortisol values. This suggests that in these patients, there may have been less oxidative damage or that dentures played a role in preventing resorption. The prompt initiation of denture production immediately after tooth extraction can be beneficial in preventing bone resorption.Dentures have proven effective in arresting the resorption process, as heightened bone resorption tendencies typically manifest within a year post-extraction. Notably, this investigation challenges the null hypothesis, given the robust inverse correlation observed between cortisol levels and bone, along with the significant disparities in cortisol levels concerning residual bone, differentiating between denture wearers and non-denture wearers.

It's worth emphasizing that this research was carried out at a single facility, specifically within a hospital university setting. This particular aspect introduces a level of complexity when attempting to arrive at definite conclusions regarding its statistical significance.Another limitation lies in the use of biomarkers, such as salivary cortisol, which primarily reflect localized phenomena pertinent to assessing residual ridge resorption (RRR). However, their overall systemic levels are subject to multifarious influencing factors. To develop a thorough comprehension of the role of oxidative stress and antioxidants in the context of residual ridge resorption (RRR), future studies should prioritize investigating the connection between local and systemic biomarkers. This research should also expand its scope by including larger sample sizes from various locations and diverse population groups.This study has furnished us with valuable insights into the initiation of stress concentration and bone loss resulting from delayed post-extraction restoration. From a clinical perspective, this investigation underscores the importance of early denture placement to impede bone resorption, enhancing the retention and support of complete dentures, and preserving bone for potential implant placement.

5. CONCLUSION:

The primary challenge faced by completely edentulous patients is the progressive issue of residual ridge resorption (RRR). While RRR is commonly regarded as a chronic, inflammatory process, it's essential to acknowledge that oxidative stress, a

natural facet of the aging and inflammatory processes, can play a role in RRR. This hints at potential links between oxidative stress and antioxidants, providing insight into the underlying mechanisms of residual ridge resorption (RRR). Our study results imply that cortisol, a common stress biomarker, can serve as an indicator for RRR in individuals who have lost all their teeth. Notably, bone loss is most significant during the first year of complete tooth loss, and the use of dentures seems to have a moderating impact on this bone resorption process.

6. CONFLICT OF INTEREST:

No conflict of interest...

Table 1 shows the comparison between two groups (patient with and without denture) based on cortisol levels

Group	N	Mean & St.Dev	SE	95%CI Upper lower	t value	*P*-value
Patient Wearing Dentures	30	52.36± 4.03	0.41	-9.42 -11.9	17.53	0.001*
Patient Without Denture	30	63.03± 10.5	0.441	-9.42 -11.9		

*statistically significant value of p <0.05 , p-value was derived from an independent t test. CI=confidence interval

Table 2 shows the comparison between two groups (patient with and without denture) based on the amount of mandibular bone height

Group	N	Mean & St.Dev	SE	95%CI Upper lower	t value	*P*-value
Patient Wearing Dentures	30	14.21± 4.03	0.34	-6.86 -5.09	13.79	0.001*
Patient Without Denture	30	8.23± 10.5	0.26	-6.86 -5.09		

*statistically significant value of p <0.05 , p-value was derived from an independent t test. SE=Standard Error , CI=Confidence Interval.

Table 3 shows the correlation between the stress marker cortisol and the amount of mandibular bone height

	Group	N	Correlation coefficient (r)	*P*-value sig. (2-tailed)	Bias	Std. Error	95% CI	
							lower	upper
Spearman's rho	cortisol	60	1.00	0.001	0.01	0.01	1.00	1.00

	bone	60	-0.811*		0.012	0.035	-0.724	-0.861
Pearson correlation	cortisol	60	1.00	0.001	0.001	0.010	1.00	1.00
	bone	60	-0.944*		0.001	0.007	-0.960	-0.930

*correlation is significant at the 0.01 level . CI=confidence interval.

Figure Legends

Figure 1: Flowchart of patient selection based on inclusion and exclusion criteria

Figure 2: The picture depicts the Orthopantomogram (OPG) image of a Completely edentulous patient.

Figure 3: strong negative correlation between stress marker cortisol and residual bone level (Pearson correlation; pearson correlation = -0.944 ; spearman correlation coefficient =-0.811)

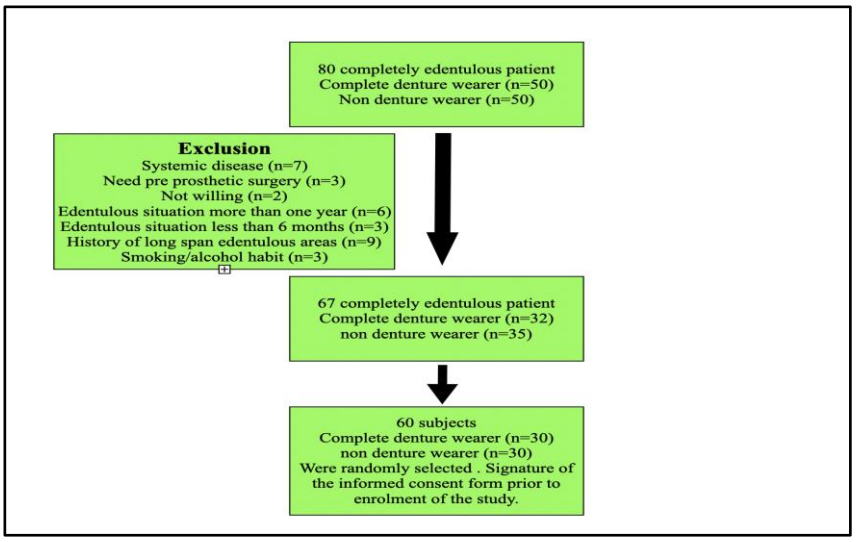

Figure 1: Flowchart of patient selection based on inclusion and exclusion criteria

Figure 2:The picture depicts the Orthopantomogram (OPG) image of a Completely edentulous patient.

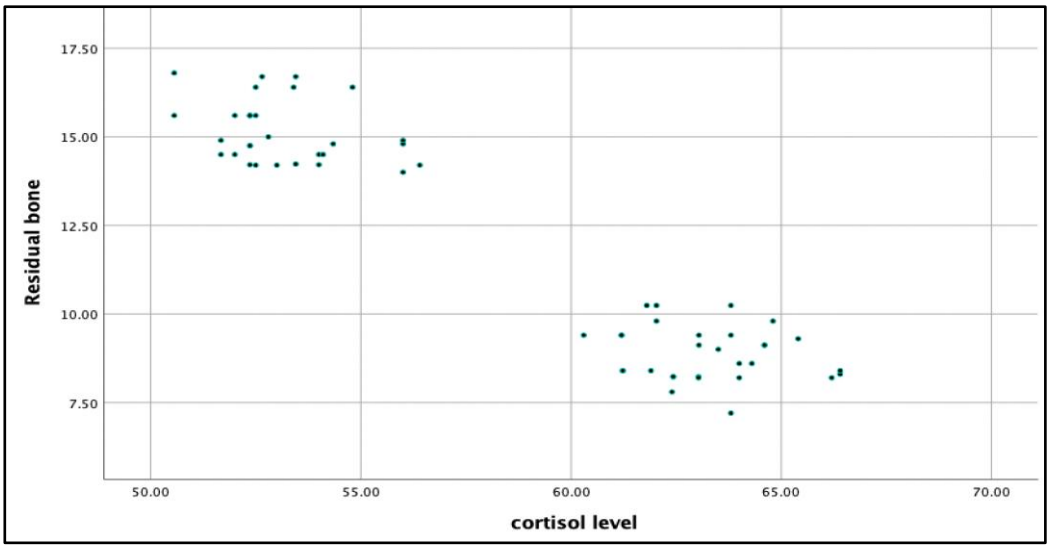

Figure 3: strong negative correlation between stress marker cortisol and residual bone level (Pearson correlation; pearson correlation = -0.944 ; spearman correlation coefficient =-0.811)

REFERENCES

[1] Gallucci GO, Hamilton A, Zhou W, Buser D, Chen S. Implant placement and loading protocols in partially edentulous patients: A systematic review. Clin Oral Implants Res. 2018 Oct;29 Suppl 16:106-134.

[2] . Carlsson GE. Symptoms of mandibular dysfunction in complete denture wearers. J Dent. 1976 Nov;4(6):265-70.

[3] . Ponnanna AA, Maiti S, Rai N, Jessy P. Three-dimensional-Printed Malo Bridge: Digital Fixed Prosthesis for the Partially Edentulous Maxilla. Contemp Clin Dent. 2021 Oct-Dec;12(4):451-453.

[4] . Agarwal S, Ashok V, Maiti S, Agarwal V. Dentists' Preference toward Fixed Versus Removable Implant Prosthesis on Edentulous Jaws to Improve Quality of Life. J Long Term Eff Med Implants. 2022;33(1):83-89.

[5] . Rajput VD, Harish, Singh RK, Verma KK, Sharma L, Quiroz-Figueroa FR, Meena M, Gour VS, Minkina T, Sushkova S, Mandzhieva S. Recent Developments in Enzymatic Antioxidant Defence Mechanism in Plants with Special Reference to Abiotic Stress. Biology (Basel). 2021 Mar 26;10(4):267.

[6] George R, Maiti S, Ganapathy DM. Estimation of L-carnitine levels in diabetic completely edentulous patients for implant diagnosis: A cross-sectional study. Dent Res J (Isfahan). 2023 Aug 28;20:96.

[7] . Shenoy A, Maiti S, Nallaswamy D, Keskar V. An in vitrocomparison of the marginal fit of provisional crowns using the virtual tooth preparation workflow against the traditional technique. J Indian Prosthodont Soc. 2023 Oct-Dec;23(4):391-397.

[8] . Gursoy UK, Könönen E. Editorial: Use of Saliva in Diagnosis of Periodontitis: Cumulative Use of Bacterial and Host-Derived Biomarkers. Front Cell Infect Microbiol. 2016 Dec 22;6:196.

[9] . Gondivkar SM, Gadbail AR, Gondivkar RS, Sarode SC, Sarode GS, Patil S, Awan KH. Nutrition and oral health. Dis Mon. 2019 Jun;65(6):147-154.

[10] Panjamurthy K, Manoharan S, Ramachandran CR. Lipid peroxidation and antioxidant status in patients with periodontitis. Cell Mol Biol Lett. 2005;10(2):255-64.

[11] . Kong Y, Olejar KJ, On SLW, Chelikani V. The Potential of Lactobacillus spp. for Modulating Oxidative Stress in the Gastrointestinal Tract. Antioxidants (Basel). 2020 Jul 10;9(7):610.

[12] . Esposito L, Tafuri S, Cocchia N, Fasanelli R, Piscopo N, Lamagna B, Eguren V, Amici A, Iorio EL, Ciani F. Assessment of living conditions in wild boars by analysis of oxidative stress markers. J Appl Anim Welf Sci. 2021 Jan-Mar;24(1):64-71.

[13] . Kim J, Yun KS, Cho A, Kim DH, Lee YK, Choi MJ, Kim SH, Kim H, Yoon JW, Park HC. High cortisol levels are associated with oxidative stress and mortality in maintenance hemodialysis patients. BMC Nephrol. 2022 Mar 8;23(1):98.

[14] . Alsaggaf A, Fenlon MR. A case control study to investigate the effects of denture wear on residual alveolar ridge resorption in edentulous patients. J Dent. 2020 Jul;98:103373.

[15]. Sharma GV, Reddy NY, Ravi R, Sreenivas B, Sridhar G, Chatterjee D, Kunwar AC, Hofmann HJ. Synthesis of C-linked carbo-β2-amino acids and β2-peptides: design of new motifs for left-handed 12/10- and 10/12-mixed helices. Org Biomol Chem. 2012 Dec 14;10(46):9191-203.

[16] Alqutaibi AY, Alnazzawi AA, Farghal AE, Bakr RM, Mahmoud II. Impact of Acrylic and Silicone-Based Soft-Liner Materials on Biting Force and Quality of Life of the Complete Denture Wearers: A Randomized Clinical Trial. J Clin Med. 2023 Mar 6;12(5):2073.

[17]. Goodacre BJ, Goodacre CJ. Additive Manufacturing for Complete Denture Fabrication: A Narrative Review. J Prosthodont. 2022 Mar;31(S1):47-51.

[18]. Denewar MM, Abdelmawgood EI, ELsyad MA, Al-Tonbary GY. Retention and Stability Characteristics of Soft-Liner and Clip Attachments Used for Bar/Implant-Assisted Mandibular Overdentures: An In Vitro Study. Int J Oral Maxillofac Implants. 2021 November/December;36(6):1095–1103.

[19]. Karunakaran N, Maiti S, Jayaraman S, Paulraj J. Assessment of Bone Turnover Markers Prior to Dental Implant Placement for Osteoporosis Patient- A Case-Control Study. Ann Dent Spec. 2023;11(2):57-61.

[20]. Zou Y, Nedelman J, Lombardi A, Pappas F, Karlsson MO, Svensson EM. Characterizing Absorption Properties of Dispersible Pretomanid Tablets Using Population Pharmacokinetic Modelling. Clin Pharmacokinet. 2022 Nov;61(11):1585-1593.