Correlation Of Salivary Malondialdehyde (MDA) And 8-Hydroxy-Deoxyguanosine (8-Ohdg) In Residual Ridge Resorption In Completely Edentulous Diabetic Patients

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ABSTRACT

Aim: The aim of this study is to correlate the salivary Malondialdehyde (MDA) and 8-Hydroxy-deoxyguanosine (8-OHdG) in residual ridge resorption in completely edentulous diabetic patients.

Materials and Method:

The study included completely edentulous diabetic and non-diabetic patients, selected based on specific inclusion/exclusion criteria. A total of 48 saliva samples were collected from 24 participants. Saliva was collected under standardized conditions and analyzed for oxidative stress markers—Malondialdehyde (MDA) using spectrophotometry and 8-hydroxydeoxyguanosine (8-OHdG) using ELISA. Residual ridge resorption was assessed radiographically using orthopantomograms and categorized per ADA (1999) criteria. Statistical analyses, including Pearson's correlation and regression models, were employed to evaluate the relationship between oxidative stress markers and resorption severity. Ethical approval and informed consent were obtained.

Statistical Analysis and Results:

48 saliva samples from diabetic and non-diabetic edentulous patients (n=24 per group). Biomarkers MDA and 8-OHdG were measured using spectrophotometry and ELISA, respectively. Statistical analysis (SPSS v23.0) included parametric and non-parametric tests based on data distribution, with significance set at $p<0.05$.

Results showed higher mean absorbance levels of MDA and 8-OHdG in non-diabetics compared to diabetics, though differences were not statistically significant. A positive correlation between MDA and 8-OHdG indicated a link between lipid peroxidation and oxidative DNA damage. These findings suggest elevated oxidative stress markers in non-diabetics, potentially reflecting adaptive metabolic responses in diabetics.

Conclusion: within the limitations of the study, Significant role of oxidative stress in residual ridge resorption (RRR), as evidenced by elevated levels of salivary biomarkers MDA and 8-OHdG in edentulous patients. Non-diabetic individuals exhibited higher levels of both biomarkers, suggesting variability in oxidative stress responses, possibly due to adaptive mechanisms in diabetic patients. A strong positive correlation between MDA and 8-OHdG further supports their reliability as salivary indicators for assessing the severity of RRR.

Introduction

Residual ridge resorption (RRR) is a progressive and irreversible process following tooth loss, leading to significant functional and aesthetic challenges in edentulous patients. The loss of alveolar bone after tooth extraction compromises denture stability, occlusion, and overall oral health. Various biological and mechanical factors influence RRR, including local inflammatory responses, genetic predisposition, and systemic conditions such as osteoporosis and diabetes mellitus.[1]

Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia, which adversely affects multiple physiological processes, including bone metabolism.[2] The interplay between diabetes and bone health has been extensively studied, with evidence suggesting that chronic hyperglycemia contributes to delayed bone healing, reduced bone mineral density, and increased susceptibility to fractures.[3] In edentulous patients, diabetes accelerates RRR through mechanisms that involve oxidative stress, chronic inflammation, and impaired bone remodeling.[4]

Oxidative stress plays a crucial role in bone degradation, particularly in individuals with metabolic disorders such as diabetes. The excessive production of reactive oxygen species (ROS) overwhelms the body's antioxidant defenses, leading to lipid peroxidation, protein oxidation, and DNA damage.[5] Among the key biomarkers of oxidative stress, malondialdehyde (MDA) is widely recognized as an indicator of lipid peroxidation, while 8-hydroxy-deoxyguanosine (8-OHdG) serves as a marker of oxidative DNA damage. Elevated levels of these biomarkers have been associated with various pathological conditions, including osteoporosis, periodontitis, and diabetic complications.[6] In diabetic patients, where oxidative stress and bone resorption are both exaggerated, salivary MDA and 8-OHdG may also reflect systemic metabolic dysfunctions contributing to oral precancerous conditions.[7]

Despite extensive research on the impact of diabetes on systemic bone health, limited studies have specifically investigated the relationship between oxidative stress biomarkers and RRR in edentulous diabetic patients. Given the rising prevalence of diabetes and its related complications, exploring the role of oxidative stress in residual ridge resorption (RRR) could offer important insights into emerging diagnostic and therapeutic approaches. The ability of biomarkers like MDA and 8-OHdG to reflect bone resorption, DNA damage, and chronic inflammation makes them valuable tools not only for monitoring the progression of RRR but also for aiding in the early detection of malignancy, particularly when combined with other clinical and laboratory investigations.

Methodology

The Study was conducted involving completely edentulous diabetic and non-diabetic patients. Participants were recruited from d, and eligibility criteria included a confirmed diagnosis of diabetes for the experimental group and the absence of systemic diseases for the control group. Unstimulated saliva samples were collected using standardized protocols and analyzed for MDA and 8-OHdG levels using spectrophotometric and enzyme-linked immunosorbent assay (ELISA) methods. Residual ridge resorption was evaluated through panoramic radiographs, with measurements taken at predetermined reference points. Statistical analyses, including Pearson’s correlation and regression models, were used to determine associations between oxidative stress markers and RRR severity. Ethical approval was obtained, and informed consent was secured from all participants.

Materials and Methods

The study was conducted in the Department of Prosthodontics and Crown & Bridge and the Centre for Advanced Research Lab, I.T.S. Dental College, Ghaziabad. Ethical clearance was obtained, and informed consent was taken from all participants. A total of 48 samples were collected from 24 patients based on predefined inclusion and exclusion criteria. The selected participants were divided into two main groups: diabetic and non-diabetic patients. The salivary biomarkers Malondialdehyde (MDA) and 8-hydroxydeoxyguanosine (8-OHdG) were analysed.

Radiographic evaluation of residual ridge resorption was performed using preoperative orthopantomograms (OPGs). The mandibular bone height was measured at its minimum vertical dimension

and classified according to the American Dental Association (1999) criteria: Class I (>21 mm), Class II (16–20 mm), Class III (11–15 mm), and Class IV (≤10 mm).

Saliva samples were collected following a standardized protocol. Patients were instructed to refrain from food and beverages for two hours before collection. They rinsed their mouths with distilled water, and approximately 2 mL of unstimulated saliva was collected using the spitting method into labeled Eppendorf tubes. Samples were immediately centrifuged for 10 minutes at 4°C to remove cell debris. The supernatant was aliquoted and stored at -8°C until analysis.

Biomarker Analysis

- MDA Estimation: A spectrophotometric method was used to assess lipid peroxidation levels. The sample was mixed with phosphate-buffered saline (pH 7.4) and butylated hydroxytoluene, followed by the addition of trichloroacetic acid. After incubation on ice, the samples were centrifuged, and the supernatant was reacted with EDTA and thiobarbituric acid in NaOH. The final mixture was heated in boiling water for 15 minutes, cooled, and absorbance was measured at 532 nm using a spectrophotometer.
- 8-OHdG Estimation: Levels were measured using a competitive ELISA kit. The process included standard curve preparation, sample incubation with biotinylated antibodies, sequential washing steps, and the addition of streptavidin-HRP solution. After further incubation and washing, TMB substrate solution was added, and the reaction was stopped using the stop solution. Absorbance was recorded at the specified wavelength using the ELISA Reader.

Figure 1: Collected Salivary samples in Eppendorf tubes of 24 patients

Figure 2: Reagents for ELISA Kit

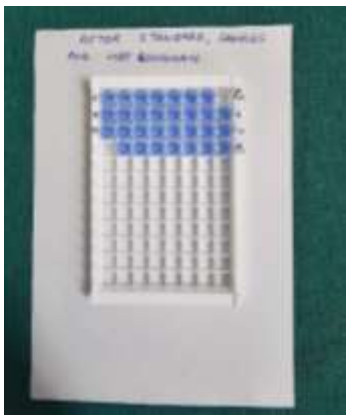

Figure 3: After standard solution, samples and HRP

figure 4: After substrate

Figure 5: After stop solution

Figure 6: Microplate ELISA reader

Figure 7: Spectrophotometer

Res

The present study was conducted in the Department of Prosthodontics and Crown & Bridge and the Centre for Advanced Research Lab, I.T.S. Dental College, Ghaziabad. A total of 48 saliva samples were collected and divided into two groups (n=24): diabetic and non-diabetic. Each group was further subdivided based on the biomarker analyzed. ELISA was used for 8-OHdG detection, while a spectrophotometer assessed MDA levels.

Statistical analysis was performed using SPSS (version 23.0). For normal data, parametric tests included ANOVA with Tukey HSD for intergroup comparison and paired t-test for intragroup analysis. For non-normal data, non-parametric tests included the Kruskal-Wallis test with Mann-Whitney U test for intergroup comparisons and the Wilcoxon signed-rank test for intragroup analysis. Statistical significance was set at $p<0.05$.

Results showed that non-diabetic patients had higher mean absorbance levels for both MDA (1.1687) and 8-OHdG (1.59167) compared to diabetic patients (MDA: 0.9676, 8-OHdG: 1.36142). Standard deviations indicated moderate variability, with non-diabetics showing greater dispersion in MDA levels. Correlation analysis revealed a positive relationship between MDA and 8-OHdG in both groups, indicating that as lipid peroxidation increases, oxidative DNA damage also rises. Scatter plots demonstrated an upward trend, supporting this association.

Graphical analysis confirmed that non-diabetic patients exhibited higher mean absorbance levels for both biomarkers. An independent t-test showed no statistically significant difference between diabetic and non-diabetic groups for either 8-OHdG or MDA, as Levene's test confirmed homogeneity of variances.

These findings suggest that oxidative stress biomarkers are elevated in non-diabetic individuals, possibly due to adaptive metabolic responses in diabetics.

Discussion

The findings of this study reinforce the role of oxidative stress in the pathophysiology of RRR, particularly in diabetic patients. The elevated levels of MDA and 8-OHdG suggest that oxidative damage accelerates bone resorption, potentially exacerbated by chronic hyperglycemia and impaired antioxidant defense mechanisms in diabetes. These results align with previous research indicating that increased oxidative stress is a major contributor to bone loss in metabolic disorders. The strong correlation between oxidative stress markers and RRR highlights the potential of saliva-based diagnostics in monitoring bone health in edentulous individuals. However, the cross-sectional nature of this study limits causal inferences, and further longitudinal research is needed to establish the temporal relationship between oxidative stress and RRR progression. Additionally, factors such as medication use, dietary habits, and genetic predisposition should be considered in future investigations.

Residual ridge resorption (RRR) is a multifactorial process characterized by progressive alveolar bone loss post-tooth extraction. This poses challenges in prosthodontics, affecting denture retention, stability, function, and aesthetics.[1] Mandibular resorption occurs faster than maxillary resorption, reducing bone height and complicating denture base stability. Excessive resorption can thin the mucosa, increasing trauma risk.

RRR is influenced by local and systemic factors. Occlusal forces, even without systemic bone loss, contribute to resorption.[2] Long-term denture wear exacerbates RRR due to mechanical stress.[7] Genetic predisposition, hormonal changes, and nutritional deficiencies (calcium, vitamin D) contribute to bone loss. Aging, smoking, alcohol, periodontitis, systemic diseases (diabetes, rheumatoid arthritis), medications (corticosteroids, bisphosphonates), parafunctional habits, and ill-fitting dentures further accelerate ridge resorption.[6]

Accurate mandibular height measurement is crucial for prosthetic planning. Various methods assess bone resorption. Cone Beam Computed Tomography (CBCT) provides detailed 3D imaging, while cephalometric analysis monitors changes over time. OPG or panoramic radiographs, used in this study, measured the least vertical height of the alveolar ridge.[9]

Diabetes mellitus is linked to increased inflammation and oxidative stress, accelerating bone resorption.[5] Oxidative stress, caused by an imbalance between reactive oxygen species (ROS) and antioxidant defenses, leads to lipid peroxidation, protein oxidation, and DNA damage, promoting osteoclastic activity.[10] This study assessed salivary biomarkers Malondialdehyde (MDA) and 8-Hydroxy-deoxyguanosine (8-OHdG), which indicate oxidative stress, inflammation, and bone resorption, and may also serve as early indicators of precancerous changes in high-risk edentulous patients.[11] Saliva was chosen as a non-invasive, cost-effective diagnostic medium.

MDA, a biomarker of lipid peroxidation, is associated with RRR via oxidative damage and inflammation.[12] 8-OHdG, a marker of oxidative DNA damage, reflects ROS-induced apoptosis in bone cells, accelerating resorption.[13] Elevated 8-OHdG levels have been observed in patients with advanced periodontal destruction and alveolar bone loss.[14] This study explored correlations between lipid peroxidation and oxidative DNA damage in RRR.

Diabetic patients were included due to heightened oxidative stress. Studies show diabetics exhibit higher MDA levels due to persistent hyperglycaemia, ROS overproduction, and impaired antioxidant defenses.[15] However, this study found non-diabetics had higher MDA and 8-OHdG levels, possibly due to adaptive metabolic responses or antioxidant effects of antidiabetic medications like metformin.[13] and other factors, such as disease duration, glycemic control, inflammation, lifestyle, and dietary habits, may influence biomarker levels.[16]

MDA and 8-OHdG detection methods include Thiobarbituric Acid Reactive Substances (TBARS) assay, High-Performance Liquid Chromatography (HPLC), Gas Chromatography-Mass Spectrometry (GC-MS), and Enzyme-Linked Immunosorbent Assay (ELISA).[17] This study employed TBARS for MDA and ELISA for 8-OHdG due to their reliability and quantitative accuracy.[18]

The study's null hypothesis, stating no correlation between salivary MDA and 8-OHdG in RRR among diabetic and non-diabetic patients, was rejected. A significant positive correlation ($p < 0.05$) was observed, indicating that increased MDA levels correspond with elevated 8-OHdG levels. Despite diabetes being associated with oxidative stress, lower biomarker levels in diabetics suggest metabolic adaptations or the effects of medications.[19] Similar findings were reported by Spoto et al. (2025)[20], showing higher 8-OHdG levels in diabetic kidney disease rather than diabetes itself.

Contrasting studies suggest prolonged diabetes increases 8-OHdG and MDA levels due to sustained oxidative stress.[21] Additionally, chronic periodontitis and insulin resistance contribute to elevated oxidative stress markers.[22] Findings align with Kaur et al. (2016)[23] and Khurshid et al. (2018)[24], supporting salivary MDA and 8-OHdG as diagnostic indicators for bone loss.

Future RRR research should integrate advanced diagnostic techniques such as proteomics and genomics to identify broader biomarkers. Longitudinal studies may provide insights into RRR progression and intervention efficacy. Personalized treatment based on biomarker profiles could improve outcomes. Chairside salivary diagnostic tools may facilitate early detection and monitoring, enhancing clinical management.

Clinically, regular monitoring of salivary biomarkers could enable early RRR detection and timely interventions, improving prosthetic success and patient quality of life. Public health initiatives could target RRR prevention, particularly in aging populations. Advancements in salivary diagnostics may refine early detection methods and drive therapeutic innovations in dental and systemic health.

This study has certain limitations, A small sample size affects generalizability. Other systemic conditions, medications, and lifestyle factors were not comprehensively analyzed. Salivary biomarker levels vary with hydration, diet, and collection timing, necessitating standardized protocols. A cross-sectional design limits causal inferences, highlighting the need for longitudinal studies. While an association between biomarkers and RRR was established, further molecular and clinical research is required to confirm causative mechanisms.

Conclusion:

1. Oxidative stress plays a key role in RRR, with elevated MDA and 8-OHdG levels in edentulous patients.
2. Non-diabetic patients showed higher MDA and 8-OHdG levels, indicating variability in oxidative stress response.
3. A strong correlation between MDA and 8-OHdG suggests their reliability as salivary biomarkers for RRR assessment.

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