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Measurement of Salivary Lipid Peroxidation in Periodontitis Patients with or Without Diabetes Mellitus

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Authors' contributions

This work was carried out in collaboration among all authors. Author VPRBRR designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft manuscript. Authors MJ and SJ managed the analyses of the study and author NDJ managed the literature searches. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aim: The aim of the present study is to assess the association of lipid peroxidation in the saliva of healthy and periodontitis patients with or without diabetes mellitus.
Study Design: Cross-sectional study.
Materials and Methods: The study was conducted in the subjects who visited the University dental hospital as outpatients from October 2020 to January 2021.The subjects were categorized into 3 groups: periodontally healthy (Group a), Periodontitis with diabetes mellitus (Group b) and Periodontitis only (Group c)
Results: Lipid peroxidation levels were found to be significantly higher ($p<0.021$ which is statistically significant) in patients with periodontitis and diabetes mellitus (108 $\pm$ 7.1) when compared to patients with periodontitis only (77 $\pm$ 3.5) and healthy group (66$\pm$5.6).

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Conclusion: The study concluded that lipid peroxidation levels are elevated in patients with periodontitis and diabetes mellitus compared to periodontitis only and also when compared to periodontal health which indicate that lipid peroxidation levels could serve as a biomarker for periodontitis in diabetic patients.

Keywords: Biomarker; diabetes, lipid peroxidase; novel method saliva; periodontitis.

1. INTRODUCTION

Oral health is of utmost importance to a human's quality of life [1]. Oral structures include hard tissues and soft tissues. Hard tissues include enamel, dentin cementum and alveolar bone. Soft tissue includes the gingiva (marginal gingiva, attached gingiva and buccal mucosa). Maintenance of oral health is most vital as it may lead to deterioration of the significant components. Dental caries and gingival diseases such as periodontitis are a menace worldwide [2-6].

There have been few studies enumerating that the prevalence of periodontal disease is much higher than that of dental caries. Periodontitis is an inflammatory disease and disorder which in turn affects the structures and components surrounding the teeth such as the periodontal ligament, cementum, alveolar bone and the gingival [7]. This disease in multifactorial in nature and is stated in my studies that the etiological factors are vast in number and vary often. Diabetes is also a common disease worldwide and is highly prevalent in India due to lifestyle and the carbohydrate rich diet [8–11]. It is proven in many studies that diabetes and periodontal health are interrelated but there's no proper evidence on which is the primary factor with respect to both the disease [12,13]. Currently plaque is the most common etiological factor for periodontal disease and there are many other contributing factors whose role is not identified yet.

The pathological interaction between the defense mechanism of the host and the pathogen eventually leads to the breakdown of periodontal tissue. There have been extensive studies researched for the biomarkers such as MDA, Vitamin D, neutrophil functions, pH of Saliva, prostaglandins E2, Interleukin -1 beta etc. Conventionally it is believed that plaque and calculus are the primary etiological factors leading to the disease of the periodontium but many factors could also play a role and are yet inconclusive [14].

Pathogenesis of periodontal disease has been researched by many studies. In the recent times there has been shift of attention towards the reactive oxygen species which is oxygen dependent and antioxidant activity could be involved in the pathogenesis of the periodontal disease. Reactive oxygen species comprises of oxygen derived free radicals, such as nitric oxide (NO), hydrogen peroxide (H2O2), hydroxyl (OH), Superoxide(O2) and hypochlorous acid (HOCL). During inflammatory conditions cells such as fibroblasts, vascular endothelial cells, osteoclasts and inflammatory cells produces reactive oxygen species [15–17]. They are of high toxicity as it harms both the microbial agent and as well as the extracellular structures which in turn can induce Lipid Peroxidation. Increased production of lipid peroxidation may induce oxidative stress and eventually damage the integrity of the cells. Lipid peroxidation is a resultant of oxidative stress and there are various markers to monitor this process.

Oxidative stress is usually determined by the decrease in the measurement of total antioxidant capacity, and conventionally the measurement of products of oxidative damage to DNA, proteins and DNA. This measurement of oxidative stress by measuring the products of oxidative damage is the most effective [18]. Few other studies enumerate that inflammatory conditions such as diabetes may also lead to increase in oxidative stress Till this date there has been very few studies assessing the lipid peroxidation levels and its association with periodontitis [19-38]. This study aims to assess the Measurement of salivary lipid peroxidation in periodontitis patients with or without diabetes mellitus.

2. MATERIALS AND METHODS

2.1 Study Design and Study Setting

This study was designed as a cross sectional pilot study. It was conducted in the subjects who visited a university dental hospital as outpatients. The study was conducted between October 2021 to January 2021. The subjects were divided into 3 groups: periodontally healthy (Group a),

Periodontitis with diabetes mellitus (Group b) and Periodontitis only (Group c)

2.2 Inclusion Criteria

Healthy periodontium (Group a) included patients of similar age and gender who had <10% of sites with bleeding on probing, no sites with probing depth ≥4 mm and no clinical attachment loss >2 mm. The inclusion criteria for periodontitis patients in group b and c were as follows: patients with not more than >2 teeth missing in each quadrant; >30% of periodontal sites with PD >4 mm; >20% of periodontal sites with interproximal clinical AL >2 mm; >30% of sites showing BOP.

2.3 Exclusion Criteria

Patients with completely edentulous arches, patients who has undergone periodontal therapy in the past 6 months, patients with history of smoking any form of drug, chronic alcoholic patients and patients with systemic diseases such as blood disorders which might delay the healing process of tissues.

2.4 Saliva Collection

The subjects were advised not to drink, eat and brush their teeth 12 hours prior to the saliva collection. Unstimulated saliva from the patient were collected and transferred to sterile containers. The samples were then immediately transported to laboratory.

2.5 Lipid Peroxidation Analysis in Saliva

Lipid peroxidation levels in saliva samples were measured in duplicate using a commercially available Malondialdehyde (MDA) enzyme linked immunosorbent assay (ELISA) Kit procured from Abbkine Scientific Co., Ltd, China as per the manufacturer protocols. This assay is used to quantitatively analysis using sandwich enzyme immunoassay technique. The samples were diluted with calibrator diluent provided with a ratio of 1:4 and the assay was performed according to the instructions. Standards were included and all results were read as the value of optical density set to 450 nm. The intra and inter assay coefficient variance (CV) was found to be ＜11% and <9%.

2.6 Statistical Analysis

The data was then input in SPSS 26.0 and the triplicate analysis was performed. Results of the experiments performed on control subjects were expressed as mean ± standard deviation. Results were analyzed statistically by a one-way analysis of variance (ANOVA) and significant differences between the mean values were measured using Newman-Keuls multiple comparison test using Graph Pad Prism version 5. The results with the $p < 0.05$ level were considered to be statistically significant.

3. RESULTS AND DISCUSSION

It was evident that lipid peroxidation levels were found to be significantly higher ($p<0.021$ which is statistically significant) in patients with periodontitis and diabetes mellitus (108 $\pm$ 7.1) when compared to patients with periodontitis only (77 $\pm$ 3.5) and healthy group (66$\pm$5.6). There have been very few studies associating lipid peroxidation levels with periodontitis and diabetes mellitus [39].

The present study describes the association of lipid peroxidation levels in periodontitis patients and also its relation to patients with diabetes mellitus. The test done with ELISA showed that the lipid peroxidation levels increased to the greatest level in periodontitis patients who were also affected with diabetes mellitus [40]. The results showed a positive correlation and high level of significance when compared between the patients with periodontal health and the patients with periodontitis and diabetes mellitus. Also, relatively significant results were observed in comparison between the patients with periodontal health and patients affected with periodontitis ($p<0.05$ proving significant).

There have been previous studies establishing the association between diabetes mellitus and periodontitis, yet our study is one of a kind as there has been no previous studies associating the lipid peroxidation levels in periodontitis patients with an inclusion of diabetes group.

Other studies also showed similar results where the lipid peroxidation levels were high in the group of patients with periodontal disease [41,42]. This is in accordance with our study and it is well established that inflammatory conditions such as periodontal diseases or diabetes mellitus and oxidative stress levels are highly associated

Table 1. Comparison of lipid peroxidation levels among 3 groups (periodontitis patients- P, patients with periodontitis along with diabetes mellitus- P+DM and patients with periodontal health). The values are expressed in µM

GROUP	Periodontal health	P + DM	P	*P value*
Lipid Peroxidation (µM)	66±5.6	108±7.1	77±3.5	P<0.021

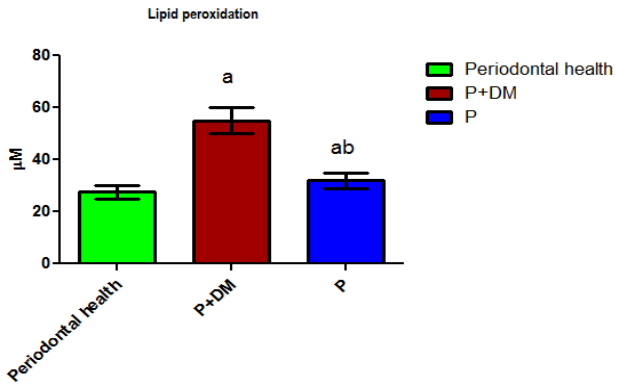

Fig. 1. Bar chart shows the assessment of salivary lipid peroxidation levels among periodontal health, periodontitis with diabetes mellitus and periodontitis only. The levels of salivary TGF-beta were assessed by Enzyme linked immunosorbent assay method. Significance at *P*<0.05, a - compared with periodontally healthy group. b - compared with periodontitis with diabetes mellitus

however there is still less evidence on which is the primary causative factor, Future longitudinal studies could be conducted to find out the primary etiology.

Our study had few limitations such as samples of different geographical locations where people may belong to the same ethnic group but not of the same age group.

4. CONCLUSION

The study concluded that lipid peroxidation levels are elevated in patients with periodontitis and diabetes mellitus compared to periodontitis only and also when compared to periodontal health which indicate that lipid peroxidation levels could serve as a biomarker for periodontitis in diabetic patients. Thus, estimation of lipid peroxidation levels may potentially aid in distinguishing healthy state from diseased conditions and also monitoring the periodontal disease activity in diabetes patients. Further studies are required with larger samples to prove lipid peroxidation levels as a standard salivary biomarker for periodontal disease.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

As per international standard or university standard, patient's written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

This cross-sectional study was carried out after obtaining approval from the institutional ethical review board.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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