Assessment of 8-Hydroxy 2-Deoxyguanosine as salivary biomarker for periodontitis patients with or without diabetes mellitus

ABSTRACT

Aim: The aim of this study is to assess the levels of 8-hydroxy-2 deoxyguanosine as a salivary biomarker for periodontitis with or without diabetes mellitus.
Study design: Cross-sectional study
Materials and methods: 30 patients were included in the study and were categorised into three groups: periodontal health (Group a), patients with periodontitis without diabetes mellitus (Group b) and patients with periodontitis only (Group c). Unstimulated salivary samples were taken. The OHdG concentration was evaluated using Sandwich-enzyme linked immunosorbent assay by commercially available human OHdG 96 well ELISA kit. The data were statistically analysed by One-Way -ANOVA. The Newman-Keuls multiple comparison test was used to test the significance at the levels of $p<0.05$.
Results: Salivary OHdG concentration in periodontal health, periodontitis with diabetes mellitus (P+DM), Periodontitis only (P) were analysed. Salivary OHdG concentration was found to be significantly higher ($p<0.05$) in periodontitis with diabetes mellitus (38±2.82 ng/L) when compared with periodontitis only (20.5±2.12 ng/L) and also when compared with healthy controls (11.5±2.12 ng/L).
Conclusion: Diabetic patients with periodontitis have increased 8 hydroxy 2 deoxyguanosine than non-diabetic counterparts. Thus salivary 8 hydroxy 2 deoxyguanosine is an effective non-invasive biomarker to assess periodontitis among patients.

Keywords: deoxyguanosine, diabetes, innovative technology, periodontitis

1. INTRODUCTION

Periodontal disease (PD) is a chronic inflammatory disorder that affects 10–15% of the world population and is considered the greatest cause of tooth loss, causing damage to all the structures that support the teeth: periodontal ligament, root cement, alveolar bone, and gingival tissues [1–4]. Its clinical classification is based on the presence or absence of signs of inflammation, periodontal pocket depth, gingival attachment loss, and bone loss [5–7].

As it progresses, neutrophils at the inflammatory site increase and, associated with macrophages, produce cytokines such as tumour necrosis factor alpha (TNF-α), interleukin-1 (IL-1), and prostaglandins[8]. During this inflammatory process, fibroblasts are stimulated by interleukin-1 and extracellular matrix metalloproteinases (MMPs) are secreted, particularly collagenase produced by polymorphonuclear neutrophils[9].

MMPs cause collagen degradation and TNF-α is responsible for increased osteoclast activity, leading to bone resorption. In addition, T-lymphocytes secrete the receptor activator of nuclear factor κB ligand (RANKL), which, in turn, is involved in osteoclast activity, ending in bone loss. Polymorphonuclear lymphocytes (PMNLs) are believed to produce active reactive oxygen species (ROS) and therefore to lead to greater production of these species[10].

Periodontal disease progression depends on immune response and the host's susceptibility. Numerous studies have pointed out that both oxidative stress and the total antioxidant capacity of the individual play an important role in the pathogenesis of periodontal diseases [11]. It has been shown that reduced antioxidant concentrations in the gingival crevicular fluid (GCF) help to increase the damage to the gums and surrounding structures caused by the action of the neutrophils. In the same way, several recent studies have shown that chronic periodontal disease is associated with hyperreactive neutrophils that have increased the production of reactive oxygen species as a response to stimulation of the Fc-gamma receptor.

Oxidative stress is defined as the state in which the balance between prooxidants and antioxidants in the organism is disturbed [12–14]. This imbalance is caused by an excess of reactive oxygen species, free radicals, and other reactive molecular species and/or by a deficiency in the antioxidant mechanisms arising from direct or indirect damage to the tissues.

One of the most important markers of oxidative stress is 8-hydroxy-2′-deoxyguanosine (8-OHdG), which is formed through oxidation of guanine from damaged DNA. Numerous studies have observed higher 8-OHdG levels in the saliva of subjects with periodontal disease than in that of healthy subjects, showing that this marker is correlated with increased ROS production during periodontal inflammation[15,16]. In the same way, its levels fall when periodontitis patients receive successful anti-inflammatory treatment. Almerich-Silla et al showed a high correlation between the presence of periodontal bacteria and the levels of 8-OHdg in saliva, which they found to be far higher than those of other oxidative stress markers.

During chronic inflammation, not only do reactive oxygen species increase in the affected tissues but a reduction in antioxidant levels is also observed. Antioxidants can be defined as substances which, at low concentrations with respect to the oxidisable substrate, significantly reduce or inhibit oxidation of that substrate[17,18]. They combat oxidative damage through direct elimination of ROS and repair of the damage caused by these detrimental agents. Antioxidants also act by downregulating some redox-sensitive proinflammatory gene transcription factors and, simultaneously, regulating inflammatory gene transcription factors.

Antioxidants are classified according to their mode of action. The preventive antioxidants include superoxide dismutase (SOD) enzymes, catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), and DNA repair enzymes.

Saliva has an important role as a tool for diagnosing and predicting periodontal diseases. Saliva is considered as an accessible tool which contains components derived from the oral mucus surfaces, gingival crevices, and tooth surfaces. It contains microorganisms that colonise the mouth, and other exogenous substances, and can therefore provide a picture of the host's relation to the environment.
Our team has extensive knowledge and research experience that has translate into high quality publications.[19–31],[32–36] [37] [38].The aim of this study is to assess the levels of 8-hydroxy-2 deoxyguanosine as a salivary biomarker for periodontitis with or without diabetes mellitus.

2. MATERIALS AND METHODS

Patients aged 30 to 60 years visiting the department of Periodontics, Saveetha dental college and hospitals, Chennai from December 2020 to February 2021 were evaluated. Thirty patients (15 males, 15 females) were included in the study. They were categorised into three groups: periodontal health (Group a), patients with periodontitis without diabetes mellitus (Group b) and patients with periodontitis only (Group c).
The inclusion criteria for the Periodontitis patients include moderate to severe periodontitis not >2 teeth missing in each quadrant; greater than or equal to 30% of periodontal sites with periodontal pocket depth greater than or equal to 4 mm; greater than or equal to 20% of periodontal sites with interproximal clinical attachment loss >2 mm; greater than or equal to 30% of sites showing bleeding on probing; and radiographic evidence of bone loss visible in posterior vertical bitewing films.
Exclusion criteria for periodontitis group were individuals who had undergone periodontal treatment in the last 6 months; history of medications (antibiotics or anti-inflammatory drugs) in the last 6 months; smoking

or use of tobacco in any form; history of alcoholism; betel nut users; history of known systemic diseases that would alter the healing response of the oral tissues; acute periodontal conditions, such as periodontal abscess and acute necrotizing gingivitis; and detection of any obvious oral mucosal lesion.

2.1 Saliva collection

Participants were instructed to refrain from certain practices such as eating, drinking, and practicing oral hygiene procedures 12 hours before saliva collection. Whole unstimulated saliva was collected from all patients and the collected samples were immediately transported to the laboratory, where it was centrifuged at 5,000 rpm for 10 minutes and the clear supernatants were stored in aliquots at -70°C. The assay was performed within 3 months of collection.

2.2 Salivary OHdG analysis in saliva

Salivary OHdG levels were measured in duplicate using a commercially available 8-Hydroxy-desoxyguanosine (8-OHdG) ELISA Kit procured from Abbkine Scientific Co., Ltd, China as per the manufacturer protocols. This assay is used to quantitatively analyse the OHdG levels 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% .

2.3 Statistical Analysis

The triplicate analysis results of the experiments were expressed as mean ± standard deviation and the data were analyzed statistically by 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.

4. RESULTS AND DISCUSSION

Salivary OHdG concentration in periodontal health, periodontitis with diabetes mellitus (P+DM), Periodontitis only (P) were analysed. Salivary OHdG concentration was found to be significantly higher ($p<0.05$) in periodontitis with diabetes mellitus (38±2.82 ng/L) when compared with periodontitis only (20.5±2.12 ng/L) and also when compared with healthy controls (11.5±2.12 ng/L) (Figure 1 and Table 1). From the above results it can be seen that OHdG was significantly higher in periodontal patients and patients with diabetes mellitus.

Periodontitis is an irreversible inflammatory disease that affects the tissues supporting the teeth. Once initiated, it progresses with the loss of collagen fibres and of attachment to the surface of the cement, apical migration of the pocket epithelium, and resorption of the alveolar bone. If not treated at an early stage, the disease advances to progressive destruction of the bone, leading to movement of the teeth and their subsequent loss[39].

Inflammatory and immune reactions to the bacterial plaque perform the leading roles in the pathogenesis of periodontitis. Most of the tissue destruction is considered to be the result of impairment of the inflammatory and immune response to this microbial plaque, causing the liberation of neutrophils, reactive oxygen species, and enzymes.

A large number of distinct types of bacteria with different pathogenicity increase periodontal inflammation. ROS are related to PMN action in the destruction of periodontal pathogens. This rise of ROS levels by PMNs would lead to tissue degeneration and a worse status of periodontal disease. Salivary stress parameters of 8-OHdG are correlated with periodontal disease and *Porphyromonas gingivalis* and its genotypes fimA II and Ib, *Treponema denticola, Aggregatibacter actinomycetemcomitans*, and *Tannerella forsythia*[40,41].

Numerous studies have pointed out that both oxidative stress and the individual's total antioxidant capacity are disturbed in subjects with periodontal disease, showing the existence of a direct association between the rise in reactive oxygen species and the fall in total antioxidant capacity in the pathogenesis of periodontal disease. [42,43] The imbalance between oxidants and antioxidants have been related with the destruction of the periodontium during inflammatory periodontal

The use of some antioxidants (lycopene and vitamin E) as periodontal treatment has the potential to improve periodontal clinical parameters; nevertheless, the role of antioxidant/oxidative stress parameters needs further investigations [44].

Miricescu et al. studied the relations between the antioxidant defense system of saliva and the levels of reactive oxygen species (ROS) in patients with chronic periodontitis and in subjects free of periodontal disease.They observed significantly higher ROS values in the chronic periodontitis group than in the control group and significantly lower levels of certain antioxidants such as uric acid, TAC, and Gpx.

8-Hydroxy-2′-deoxyguanosine (8-OHdG) is formed through oxidation of guanine from damaged DNA, causing severe damage to periodontal tissues. Higher salivary 8-OHdG reflect increased oxygen radical activity during periodontal inflammation. The present review has shown that although 8-OHdG is present both in subjects with no periodontal disease and in those with this illness, its levels are significantly higher in the saliva of the periodontal disease patients Takane et al., Sawamoto et al., Sezer et al., Komatsu et al., Almerich-Silla et al., and Zamora-Perez et al. all found the 8-OHdG levels in subjects with periodontal disease to be high, and very high compared to those in healthy controls.
The limitations of the present study include restricted geographical limit and the variation of results which may be caused due to the sample selection, diabetic status, salivary collection and transport etc.

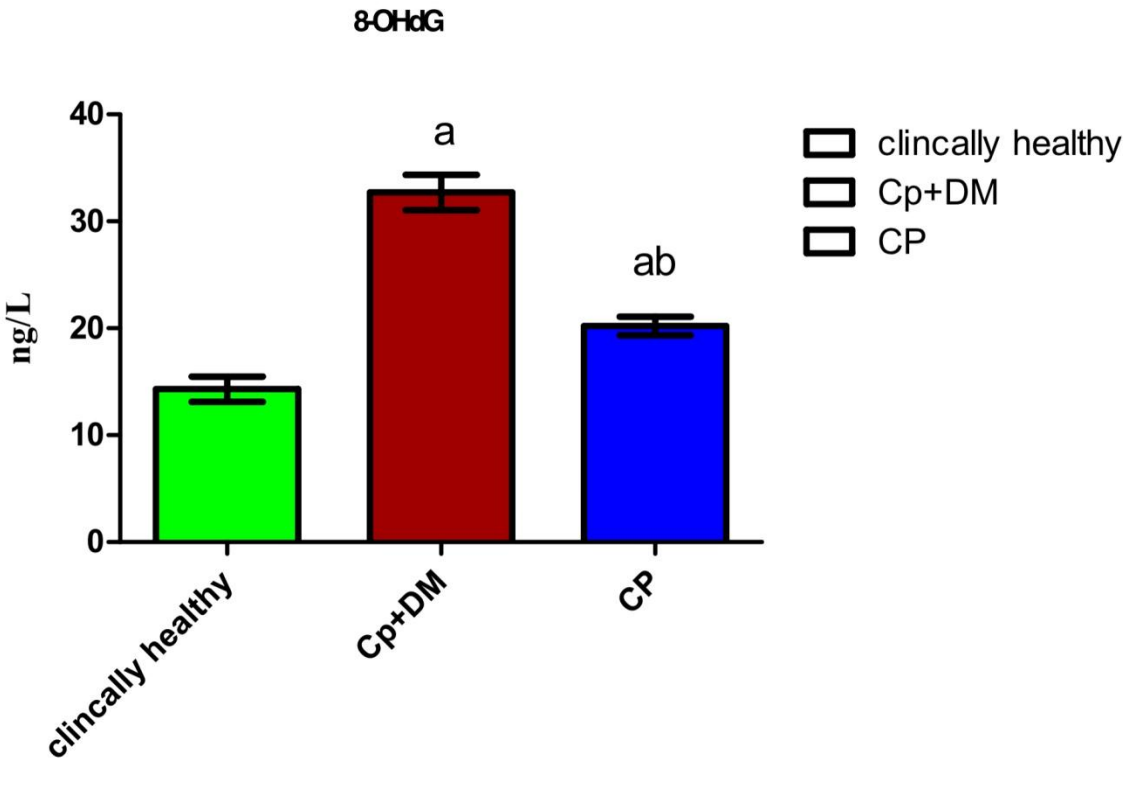

Fig. 1. represents the salivary OHdG concentration in periodontal health, periodontitis with diabetes mellitus (P+DM), Periodontitis only (P). The concentration of OHdG was measured by ELISA method. Each bar represents the mean ± SD of 3 observations. The significance was considered at the levels of p<0.05. a-compared with periodontal health (P); ab-compared with P and P+DM

Table 1 represents the OHdG level in healthy patients, Periodontally compromised patients and patient with periodontitis and diabetes. The p value was <0.05 suggesting that there is significant difference in the level of OHdG among the different groups

Group	Periodontal health (Group A)	P+Diabetes mellitus (Group B)	Periodontitis (P) (Group C)	p value
8-OHdG(ng/L)	11.5± 2.12	38± 2.82	20.5± 2.12	0.0035

3. CONCLUSION

It can be concluded that diabetic patients have more periodontal tissue destruction and increased 8 hydroxy 2 deoxyguanosine than non-diabetic counterparts. Thus 8 hydroxy 2 deoxyguanosine is an effective salivary biomarker to assess periodontitis among patients.

CONSENT

It is not applicable

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. 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.

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