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Assessment of Some Biochemical Oxidative Stress Markers in Type II Diabetics and Non-diabetics with Chronic Periodontitis

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Authors' contributions

This work was carried out in collaboration between all authors. Author ASA designed the study, managed the literature searches, wrote the protocol, obtained the data and wrote the first draft of the manuscript. Authors MGK and ANS supervised and guided the study. Authors RK and TB managed the clinical evaluation of the study. Author MD helped in biochemical evaluations and helped in the statistical analysis. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Comparative assessment of some biochemical oxidative stress markers in Type II diabetics and non-diabetics with chronic periodontitis.
Study Design: The cross sectional study groups were clinically evaluated and their biochemical parameters were assessed and statistically compared.
Place and Duration of Study: Departments of Biochemistry and Dentistry, Grant Medical College and Sir J J Group of Hospitals, Mumbai, India, between May 2010 and July 2012.
Methodology: 168 individuals with generalized chronic periodontitis (CAL ≥ 3 mm; American

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Academy of Periodontology criteria) were divided them into non-diabetics (F Glucose ≤ 5.5 mmol/L; CP group, n = 86) and diabetics (F Glucose ≥ 7 mmol/L; WHO criteria, CPDM group, n = 82). The diabetic status was ascertained by measuring the Fasting plasma glucose (F Glucose). Apparently healthy individuals (CAL ≤ 3 mm and F Glucose ≤ 5.5 mmol/L; C group, n = 120) were recruited as controls. The periodontal status for the control and the aforementioned study groups was evaluated by measuring gingival index (GI), plaque index (PI), papillary bleeding index (PBI), probing depth (PD) and clinical attachment level (CAL). The biochemical oxidative stress markers namely total antioxidant capacity (TAC), superoxide dismutase (SOD), glutathione peroxidase (GPx), vitamin C, malondialdehyde (MDA) were estimated.

Results: The clinical periodontal parameters were significantly (p≤0.05) higher in CPDM than CP, and both the diseased group v/s controls. The biochemical markers also showed similar trend as that of clinical parameters. TAC, GPx, vitamin C got significantly reduced and SOD, whereas, MDA got significantly increased.

Conclusion: The individuals with diabetes and chronic periodontitis may be at a higher risk of oral and systemic oxidative stress damage compared to non-diabetic with chronic periodontitis.

Keywords: Diabetes mellitus type II; chronic periodontitis; oxidative stress; biochemical markers.

1. INTRODUCTION

Diabetes mellitus (DM) is a group of metabolic disease characterized by hyperglycemia resulting from defects in insulin secretion, insulin action or both [1,2]. It has a global prevalence rate of 8.9% [3] and an increasing prevalence in south-east Asia including India [3,4]. Chronic Periodontitis (CP) is an inflammatory disease of the periodontium; the supportive tissue of the teeth. It is caused by group of specific microorganisms, resulting in progressive destruction of the periodontal ligament and alveolar bone with pocket formation, recession or both [5]. The relationship between diabetes (a systemic disease) and periodontitis (an oral disease) has been investigated [6] and it has showed mutual influence on each other [1,7].

Oxidative stress (OS) has been related increasingly to the onset and/or progression of growing number of human diseases [8] including diabetes [9] and periodontitis [10]. In diabetes, hyperglycemia can induce OS via several mechanisms, like glucose oxidation, the formation of advanced glycation and lipoxidation end products (AGEs/ALEs) [11]. On the other hand, OS has been linked with both onset of periodontal tissue destruction and progression of CP [12,13]. Recent research focuses on the role of reactive oxygen species (ROS), anti-oxidant (AO) systems, and products of OS in the pathology of periodontitis [14]. Thus, OS seems to be a common link between both the diseases, therefore the co-existence of some common OS pathological pathways needs further clinical investigation.

We hypothesized that individuals with DM and CP may show aggravated intensity of periodontitis and higher OS compared to non-diabetics. Therefore this study has been undertaken to comparatively assess the OS markers in CP patients with and without DM.

2. MATERIALS AND METHODS

The study was undertaken as per the approval of the Institutional Ethics Committee of Grant Medical College and Sir J J Group of Hospitals, Mumbai. A written informed consent was obtained from all the subjects enrolled in the study.

2.1 Study Groups

A total of 288 individuals who visited the department of dentistry, Grant Medical College, Mumbai, were recruited and divided into the following study groups:

Group I (C): Healthy controls; n = 120 (males = 65, females = 55), mean age = 38.5±5.5

Group II (CP): Non-diabetics with CP; n = 86 (males = 51, females = 35), mean age = 40.9±4.6

Group III (CPDM): Type II Diabetics with CP; n= 82 (males = 50, females = 32), mean age = 47.7±7.0

The patients in the study groups were clinically evaluated for CP according to the criteria of the American Academy of Periodontology (1999) [15]. It was ascertained that the patients had minimum of 20 teeth present of which at least 30% had a site with probing depth (PD) ≥5 mm and clinical attachment level (CAL) ≥3 mm. The

patients in group CP were otherwise healthy, with no history of major illness and no consumption of antioxidants, antibiotics, anti-inflammatory or any other drugs. Also, they did not receive any periodontal therapy for at least 6 months prior to the inception of the study. Individuals having past illness and undergoing any treatment, diabetics and alcoholics were excluded from the CP group. The patients in the diabetic group (CPDM) were type 2 diabetics (following WHO criteria, fasting glucose ≥ 7.0 mmol/L) [16] with an average duration of diabetes being 7.8 ± 3.2 yrs. About 50% of them reported other complications of diabetes, predominantly retinopathy and nephropathy. They were taking oral hypoglycemic agents and followed diet restriction. The individuals in the control group (Group C) were from the same geographical location with apparently good oral and systemic health and without any habits.

2.2 Clinical Measurements

The periodontal status of all individuals was evaluated by measurement of GI as developed by Loe and Silness [17], PI as described by Silness and Loe [18] and PBI developed by Muhlemann [19]. The PD and CAL measurements were done as prescribed in [20]. All clinical measurements were evaluated by a single dental professional using University of North Carolina (UNC-15) probe (Hu-Friedy, Chicago).

2.3 Sample Collection

A total of 4 ml venous blood was collected in disposable syringe from all the subjects following standard precautionary measures. Of this, 1 ml blood was transferred to sodium heparin vacutianer (VACUETTE ®) and was used for analysis of TAOC, RBC-SOD and GPx. The remaining 3 ml of blood was transferred to Serum Sep Clot Activator vacutianer (VACUETTE ®) and allowed to stand at room temperature (RT) for 30 min and centrifuged at 3,000 rpm for 20 min to obtain serum, which was stored at −4°C until further analysis. The serum was analyzed of vitamin C, MDA. All the biochemical markers were measured on calibrated semi auto analyzer BIOTRON BTR-830 ® (Ranbaxy laboratories, India).

2.4 Biochemical Studies

The plasma TAC was measured by the Ferric Reducing Ability of Plasma (FRAP) assay according to the method of Benzie FFI and Stain JJ [21]. The reaction measures antioxidant reduction of Fe^{3+}TPTZ (tripyridyltriazine) to blue colored Fe^{2+}TPTZ measured at 593 nm.

The blood RBC-SOD and GPx were measured using the RANSOD ® and RANSEL kits ® (Randox Laboratories, UK) respectively following the manufacturer's instructions [22,23].

The serum vitamin C content was measured using the dinitro-phenyl hydrazine (DNPH) method [24]. In strong acidic medium, vitamin C is oxidized to diketogluonic acid which reacts with 2,4 DNPH to form diphenylhydrazone which dissolves in strong sulphuric acid solution to produce a red colored complex which was measured at 500 nm.

The serum MDA was estimated according to the method of Kei S [25]. Lipoproteins were precipitated from the serum by adding trichloro acetic acid. 0.05 M sulphuric acid and 0.67% thiobarbituric acid (TBA) in 2 M sodium sulphate were added and the coupling of lipid peroxide with TBA was carried out by heating. The resulting chromogen was extracted in *n*-butanol whose absorbance was measured at 530 nm.

Glucose oxidase-Peroxidase (GOD-POD) kit was employed for glucose estimation ensuing manufacturer's protocol [26].

2.5 Statistical Analysis

The data was statistical analyzed using Statistical Package for Social Sciences (IBM-SPSS version 20) for MS Windows. The values on clinical parameters and biochemical markers were expressed as mean ± SD across the three study groups. The normality assumption for each clinical and biochemical parameter was tested using criteria suggested by George and Mallery [27]. Comparison of significance of difference of average of clinical parameters and biochemical markers across the three study groups was done using ANOVA with Tukey's correction for multiple group comparison. P value < 0.05 is considered to be statistically significant.

3. RESULTS AND DISCUSSION

The relationship between DM and CP has been extensively investigated, and has shown mutual influence on each other. Glycemic control appears to be an important determinant in this relationship [1,7]. Diabetes increases the risk of periodontitis and the presence of periodontitis

over time increases the risk of worsening the glycemic control, indicating a direct relationship between DM and periodontal disease [28]. In diabetes, hyperglycemia can induce OS via several mechanism like glucose oxidation, formation of AGEs-ALEs and their interaction with receptors (AGE-RAGE interaction), protein kinase C (PKC) dependent activation of NADPH oxidase in neutrophils and other phagocytes etc. [11,29]. Diabetes also leads to shift in redox balance, increased generation of reactive oxygen and nitrogen species (RONS), modification of enzymes or structural proteins by glycation, which impairs oxidant: AO balance. Besides, recently it has been suggested that acute glucose fluctuations in diabetics may directly lead to OS [30,31,32]. All these mechanisms may alter the oxidative balance in periodontium and may contribute to the exacerbated periodontal destruction in diabetes.

The above factors may contribute in the pathogenesis of both the diseases when they co-exist and may alter the clinical and biochemical outcomes in the host. The present study has observed significantly altered clinical parameters among the study groups with increasing clinical damage from healthy to diabetics with CP group. (Table 1).

Similar to our findings, studies [14,33,34] have associated altered clinical parameters in CP as compared to healthy controls. The bacterial products like lipopolysaccharides (LPS), peptides, toxins etc., leading to host inflammatory response and release of pro-inflammatory cytokines like IL-12, TNF-α, IL-1β etc. Further, discharge of antibacterial peptides, antibodies and release of collagenolytic enzymes from the host tissues results in periodontal ligament destruction and bone resorption [35]. When individuals in CPDM group were compared to those in CP group with respect to their clinical parameters, it was found that CPDM group showed significantly higher values for these markers than CP group (Table 1) except PBI, which was relatively higher in CPDM than CP, but could not reach statistical significance ($p = 0.163$). It is reported that patients with type II diabetes mellitus (T2DM) have more plaque and thus have more susceptibility for severe periodontitis and deranged clinical parameters [36] as observed in our study.

Both periodontitis and diabetes can modulate host immune response, leading to elevation of pro-inflammatory cytokines and subsequently increased OS [11]. Gingival reactive hyperemia was attenuated by periodontal disease and this effect was also remarkable in DM models. Furthermore, vascular endothelial function was decreased in DM and/or periodontal diseases animal models due to increased OS in gingival circulation [37].

One of the most common extensively studied OS markers is TAOC, which reflects full spectrum of AO against various RO and N radicals. Generally low TAOC indicates OS or increased susceptibility to oxidative damage [38]. Studies [34,39,40] have associated periodontitis with TAC and reported significantly lower values than those in controls, similar to our findings. Studies have also associated OS to CPDM, in that, when biochemical markers were compared it was seen that CPDM group showed significantly different values than CP The significantly decreased total antioxidant status (TAS), in the peripheral blood in CP may be one of the pathogenic mechanisms underlying the link between periodontal disease and several systemic diseases like diabetes [41]. Increased production of free radicals directly caused by hyperglycemia might lead to increased consumption of AOs and this leads to a decrease in AO stores [42].

AO enzymes, SOD and GPx provides protection within the cell against ROS. The function of SOD is to remove damaging ROS from the cellular environment by catalyzing dismutation of two

Table 1. Inter group comparison of the clinical parameters

Clinical parameters	Mean±SD			*P** value		
	Group C (n=120)	Group CP (n=86)	Group CPDM (n=82)	Group C vs. Group CP	Group C vs. Group CPDM	Group CP vs. Group CPDM
GI	0.67±0.11	2.39 ± 0.48	2.68 ± 0.50	0.001	0.001	0.001
PI	0.43 ±0.42	2.19 ± 0.52	2.27 ± 0.50	0.001	0.001	0.001
PBI	0.82 ±0.87	2.57 ± 0.36	2.65 ± 0.44	0.001	0.001	0.168
PD (mm)	1.73 ±0.30	5.44 ± 0.44	5.60 ± 0.49	0.001	0.001	0.024
CAL (mm)	1.83 ±0.30	7.68 ± 0.88	8.01 ± 0.99	0.001	0.001	0.024

*Values are mean ± SD. * P- values ANOVA with Tukey's correction for multiple group comparisons*

Table 2. Inter group comparison of the biochemical markers

Biochemical markers	Mean±SD			*P** value		
	Group C (n=120)	Group CP (n=86)	Group CPDM (n=82)	Group C vs. Group CP	Group C vs. Group CPDM	Group CP vs. Group CPDM
TAC(μM/L)	913.42±66.0	826.34±76.81	796.89±71.13	0.001	0.001	0.011
RBC-SOD (U/g Hb)	290.85±38.27	527.80±78.06	561.07±75.44	0.001	0.001	0.006
GPX(U/g Hb)	13.63±1.43	8.27±1.23	7.74±0.82	0.001	0.001	0.001
Vit C(μM/L)	35.59±3.87	27.57±3.59	25.09±3.88	0.001	0.001	0.001
MDA(nM/ml)	2.02±0.24	4.11±0.38	4.58±0.36	0.001	0.001	0.001
F Glucose* (mmol/L)	4.95±0.38	5.05±0.33	8.63±1.99	0.242	0.001	0.001

*Values are mean ± SD. * P- values ANOVA with Tukey's correction for multiple group comparisons. (* F Glucose= Fasting Glucose)*

super oxide radicals to hydrogen peroxide (H_2O_2); GPx reduces H_2O_2 by the oxidation of reduced glutathione [43]. Contradictory findings are repeated in the literature related to SOD activity in CP. In the present study significantly higher enzyme activity from RBC-SOD has been observed (Table 2) as also being reported by studies [14,44]. In contrast to our findings, studies [45,46] have observed significantly lower SOD activities in CP patients. The human periodontal ligament has shown to possess the enzyme SOD which offers biological protection against ROS. Bacterial LPS also stimulates superoxide release from gingival fibroblast, suggesting that the induction of SOD may represent an important defense mechanism of the fibroblast during inflammation [14]. In the present study increased RBC-SOD activity in CP supports the above findings as part of the systemic response. With respect to GPx, the present study has observed significantly lowered enzyme activity in CP group compared to in controls (Table 2) similar to the findings of studies [46,47]. However studies [48,49] have shown insignificant change in salivary GPx in CP and have observed direct proportionality in GPx activity in gingival crevecular fluid (GCF) with the severity of periodontal disease respectively. Al Rawi NH 2011 [50] have reported that OS in various pathologies (including periodontitis) does not depend on a loss or a lack of reduced glutathione (GSH) synthesis alone but a misbalance in the oxidant/reductant cycle of GSH. The depleted GSH concentration may be reflected in lowered GPx activity. AO enzymes were also documented in studies considering patients with periodontitis and diabetes. Results of studies on SOD activity in relation to periodontitis and diabetes are conflicting. Higher [14,31] and lower [9,32] SOD activity are reported in both periodontitis and diabetes. The present study has observed significantly higher SOD in patients with diabetes compared to CP group (Table 2). Salivary SOD and GR activities were significantly different in periodontitis and diabetics with periodontitis [51]. Further, SOD was significantly induced and up regulated in the poorly controlled diabetics with periodontitis [52]. Besides, it was observed that there was also significantly lower GPx activity in patients with T2DM than non-diabetes controls [9]. The present study has detected significantly lowered GPx activity in diabetes group than in CP group (Table 2). Similar observations, with decreased GPx [53] and a significantly lowered serum GSH and AO was reported in CPDM than controls [54].

Vitamin C is a low molecular weight, water soluble AO, has protective effect on maintaining tissue homeostasis by playing important role in collagen synthesis and therefore helps in maintenance of structural integrity of the connective tissue. It is required for tissue wound healing including the periodontium. It's deficiency is linked to increased permeability of gingival mucosa which allows easy passage of microbial and other noxious products into the periodontium [55]. It also has a beneficial role as radical scavenger [56]. Vitamin C, through its AO action, neutralizes OS, and in doing so may be depleted in plasma. It is therefore quite possible that periodontitis causes lower plasma vitamin C through this mechanism [57]. In our study serum vitamin C level was significantly lower in CP as compared to controls (Table 2) which was also found by [58,59]. Vitamin C might also play a critical role in the etiology and/or progression of

periodontitis in diabetes. Diabetes contributes to oxidative tissue damage and given the AO properties of vitamin C, it might act as a potential moderator in diabetes-periodontitis relationship [55,56]. Significantly lower vitamin C is reported in both periodontitis [59] and diabetes [60]. In accordance to results of our study, studies [61,62] have also reported decreased levels of vitamin C in diabetic patients with periodontitis. They have also showed that a higher levels of vitamin C is associated with clinical improvement.

One of the most damaging effects of OS is the induction of Lipid peroxidation (LPO). The cell membrane which is composed of poly-unsaturated fatty acids is a primary target for reactive oxygen attack leading to formation of lipid peroxides, which are toxic and capable of damaging most body cells. Many aldehydes are produced during the peroxidative decomposition of unsaturated fatty acids. One of them is MDA, which has been considered for a long time as the most important non-toxic and stable LPO metabolite [63,64]. MDA is one of the most frequently used indicators of LPO and may be a potential biomarker indicating OS [65]. Our finding indicates significantly higher serum MDA level in CP as compared to the healthy controls (Table 2). Many researchers [14,66,67] have also observed higher MDA levels in CP. Higher MDA level associated with increased OS is documented in both periodontitis [14] and diabetes [42,53]. Hyperglycemia in newly diagnosed patients with T2DM is associated with elevated OS through increased LPO (MDA) and depleted AO capacity [60,68]. Reshamwala SMS et al 2005 [69] reported significantly higher thiobarbituricacid reactive substances (TBARS) in erythrocytes plasma membrane of T2DM patients. The present study showed significantly higher MDA level in CPDM group compared to CP group (Table 2).

Both diabetes and periodontitis could be related by a common factor i.e. OS. Diabetes allows a pro-oxidative state in periodontal tissue, altering AO defense mechanism. On the contrary periodontitis, being a source of oxidative damage, promotes the onset of insulin resistance in a vicious cycle. Furthermore, periodontitis may influence systemic conditions by; a. increased antibody production which may react against endothelial proteins (autoimmune theory), production of inflammatory mediators in gingival tissues, their diffusion in the systemic circulation (inflammatory theory) and b. diffusion of oral pathogens or their virulence factor in systemic circulation and activating endothelial cells (bacteriological theory) [70].

Overall, the present study showed increasingly higher clinical damage and systemic OS (elevated oxidants and compromised AO) from healthy state to periodontitis (without diabetes) and periodontitis with diabetes. Further studies with a large number of biochemical markers and/or marker profiles may be incorporated to give better information about AO and oxidant status in the diseased individual. A periodontal therapy response on these markers could also be of future interest.

4. CONCLUSION

The significantly higher clinical parameters and oxidative stress markers in periodontitis individuals compared to healthy controls points to the damaging effect of this disease on oral and systemic health. When the study parameters were compared between diabetics and non-diabetics with periodontitis, diabetic patients showed more clinical damage and higher oxidative stress. This further indicates that periodontitis add an additional burden in DM.

CONSENT

All authors declare that written informed consent was obtained from the patient for publication of this manuscript.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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