Biochemical Parameters and Indicators of Oxidative Stress in Indian Periodontitis Patients

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Abstract

Periodontal disease, also known as chronic periodontitis, causes inflammation in the periodontium. Biochemical oxidative stress markers were measured and compared between patients suffering from chronic periodontitis & healthy controls& the impact of nonsurgical periodontal treatment was assessed. A total of 65 healthy volunteers and 5 patients suffering from chronic periodontitis were chosen at random to take part in the study. Periodontal health was determined by measuring clinical attachment losses, gingival score, bacterial index, and gum bleeding (CAL). Biochemical indicators included vitamin C, peroxidation, CRP, red blood cell superoxide dismutase (GPx), and total antioxidant capacity (TAOC). Non-operative periodontal therapy (scaling & root planing; SRP) was used to treat patients with chronic periodontitis, and patients were checked up on 3 months later. Patients suffering from chronic periodontitis had considerably higher levels of CRP, MDA, and RBC-SOD compared to controls, while they had significantly lower amounts of TAOC, GPx, or vitamin C. Biochemical and clinical oxidative stress markers were considerably reduced in patients treated with SRP for periodontitis. Patients suffering from chronic periodontitis have elevated levels of inflammation and oxidative stress. With SRP treatment, the systemic oxidant:antioxidant equilibrium is restored and the inflammatory burden is lessened. Periodontitis is an inflammatory disease, and SRP treatment may be helpful in its management and prevention.

Keywords: Oxidative stress, biochemical, parameters, periodontitis

INTRODUCTION

Gum disease, also known as periodontitis, causes inflammation and destroys the supporting tissues of the teeth. The subgingival biofilm is to blame, but the aberrant host reaction that promotes its growth. [2] Heart disease, obesity, osteoporosis, and unfavourable birth outcomes are just some of the systemic conditions that might arise, have risk factors that include periodontal disease [3, 4]. When there are more pro-oxidants than antioxidants, the body is under oxidative stress. An ever-expanding number of human illnesses have been connected to it [4]. Periodontal tissue loss and systemic inflammation are both associated with oxidative stress [5]. Superoxide anion, hydroxyl radicals, nitrous oxide, or hydrogen peroxide are all examples of ROS that are generated by the bacterial-host mediated route, stimulate respiratory burst production of superoxide radicals by polymorphonuclear leukocytes (PMNL). Enhanced ROS conc causes oxidative injury to periodontal tissues due to a deficiency in circulating antioxidant: Antioxidant balance [6,7].

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According to a study, smokers had a higher risk than non-smokers of developing serious forms of subgingival calculus and that tobacco use is one of the key risk variables for chronically destructive periodontal disease [8]. About three times as many smokers as non-smokers suffer from severe periodontitis [9].Tobacco use is linked to oxidative stress. Inflammatory responses to cigarette smoking are mostly caused by oxidants produced from cigarette smoke. Since both smoking or persistent periodontitis raise oxidative stress indicators, their interaction needs to be investigated further.

Increased ROS production is a characteristic of chronic and severe periodontitis [13, 14]. The possible activation of PMNLs in their physiological roles may explain the observed uptick in ROS generation. No one knows how much the production of reactive oxygen species (ROS) leads to periodontal diseases, but it is crucial to remember that numerous antioxidant organisms have indeed been involved in defence mechanisms against elevated ROS activity, and thus the effect of Ros generation shouldn't be considered in isolation and keep the host tissues in a state of delicate equilibrium [16]. A large body of research has established a connection between these chemicals and osteoclastic bone resorption [15] & periodontal tissue decay.In periodontal disease, it is well recognised that inflammation is significantly related with enhanced oxidative stress components. Therefore, they may be mediated by an unchecked inflammatory response in periodontitis [17].

The purpose of this research was to analyse the differences between healthy Indian individuals and those with chronic periodontitis by analysing their plasma for metabolic indicators & oxidative stress markers.

LITERATURE REVIEW

Patel et al. (2012) [18] focused GCF or serum eGPx values to find out what role oxidative stress plays throughout periodontal diseases and how NSPT affects them. The sample included 30 people (16 men and 14 women, aged 30–38). Group 1 consisted of healthy individuals (n=10), group 2 consisted of those with gingivitis (n=10), and Group 3 (n=10) was found to have periodontitis based on the gingival score, probing depth of penetration, or clinical attachment degree. Group 4 included participants whose periodontitis symptoms persisted despite receiving NSPT. Quantification of eGPx in GCF and serum was performed using enzyme-linked immunosorbent assays. Patients with gingivitis had an average of 22.86 ng/l of endoglin (eGPx) in their gingival crevicular fluid (GCF) and a mean of 90.44 ng/ml of eGPx in their serum, whereas those with periodontitis had a mean of 29.89 ng/l of eGPx in their GCF and a mean of 103.43 ng/ml in their serum. Concentrations of both GCF (19.41 ng/l) or blood eGPx (85.21 ng/ml) dropped noticeably after NSPT.

Dabra et al. (2012) [19] evaluated periodontal disease patients' saliva AST, ALT, GGT, ALP, or ACP activities (20 gingivitis patients or 20 periodontitis patients inside the experimental class) before and after treatment, as well as the saliva of healthy people (20 samples in the control group). Saliva from the patient, after being stimulated, was collected in a clean test tube & analysed with an Automatic Analyzer. Salivary values for AST, ALT, GGT, ALP, and ACP

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were all considerably higher in patients having gum disease compared to the control group. Within a week of regular periodontal therapy, enzyme levels declined substantially.

Allen et al. [20] looked at how based on these numbers affects the state of oxidation and inflammation and Controlling glucose in individuals who have Type 2 diabetes. Type 2 diabetes affects 20 people or periodontitis (BMI 31 + 5) had their blood drawn and their teeth looked at while they were fasting. Twenty controls having Type 2 diabetes (BMI 29 + 6) or twenty controls without diabetes (BMI 25 + 4) of similar age and sex were also studied. Fasting blood sugar, haemoglobin A1c, lipid panel, insulin sensitivity, or insulin secretion were all evaluated to confirm a diabetes diagnosis. Total leucocyte count, fibrinogen levels, and high-sensitivity C-reactive protein levels were all examined to assess inflammation (hsCRP). SPSS was used for all analyses requiring statistical significance. Protein carbonyls were found to be higher ($p = 0.007$) and pSMAC was found to be decreased ($p = 0.03$) in type 2 diabetes patients who also suffered from periodontitis. High levels for haemoglobin A1c were seen in patients who had diabetes and periodontitis (p0.002) and fasting glucose (p0.04), in addition to decreased -cell function (HOMA-; p0.01). Patients who had type 2 or periodontitis had substantially lower HDL-cholesterol levels and greater hsCRP levels compared to those without diabetes (p0.004). However, periodontitis had no impact on markers of inflammation or lipid profiles.

Parwani et al. (2011) [21] studied that saliva contains biochemical markers of inflammatory periodontal illnesses and can serve as a partial predictor of the severity of periodontal infection. The technique of saliva extraction is also easy and non-invasive. It has been hypothesised that nitric oxide (NO) in the mouth contributes to the onset of inflammatory periodontal disorders. Goals Saliva NO levels were compared between healthy participants and those with inflammatory periodontal diseases (gingivitis and periodontitis) to those of people with healthy gums and teeth. This was also repeated after the medicine was given to determine its diagnostic and prognostic value. The total number of participants in this case-control and interventional trial was 90. (30 controls, 30 gingivitis, & 30 periodontitis). Saliva samples were analysed for NO content using the Griess reaction. Patients with periodontal diseases including gingivitis and periodontitis have much greater NO levels than healthy people. There was a considerable drop in NO levels across all groups after the recovery phase. In addition, an association between probing pocket depths and NO levels in saliva was found in the periodontitis group.

Gazy et al. (2012) [22] investigated how smoking & CP may interact to affect salivary biochemical indicators. Eighty systemically healthy males were enrolled in this study. According to their periodontal health & smoking status, they were categorisedEach person's UWS (i.e., unstimulated whole saliva) was collected. During the sampling process, the quotient of saliva flow rate (FR) was calculated. Saliva was tested for its acidity, total protein content, albumin, and globulin, fucose, and C - reactive protein levels. Saliva production was not affected by the periodontal health status. Saliva pH was shown to be lower in smokers compared to nonsmokers, with no correlation to periodontal health. CP patients had increased levels of TP, Alb, &Alb/Glo ratios. Both CP & CP smokers had higher levels of salivary Glo and TF than nonsmokers, but lower levels of salivary PBF. All of these concentrations, with the exception of TP, had zero

effect on my habit of smoking. Cigarette smokers tend to have greater CRP levels than nonsmokers do, regardless of their periodontal health.

Duarte et al. (2012) [23] evaluated the transcriptional activation of antioxidant enzymes inside the gingiva of people with type 2 diabetes or chronic periodontitis who have poor control or good control of their disease (CP). Biopsies were taken from the gums of 12 people who were healthy overall and in their gums, 15 people who were healthy overall but had CP, and people with CP who have diabetes and are either well-controlled (n = 8) or poorly-controlled (n = 14). Multiple antioxidant enzymes, such as peroxiredoxin (PRDX) 1 & 2, catalase (CAT), guanylate pyrophosphate oxidase (GPX1), and superoxide dismutase (SOD) isoforms 1 and 2, had their mRNA expression levels analysed using quantitative polymerase chain reaction (qPCR). Periodontitis increased the expression of PRDX1 and GPX1 (p0.05) regardless of glucose levels. On the other hand, PRDX2 and SOD2 were only slightly affected by periodontitis, but they were strongly reported to induce when periodontitis has been combined with DM, especially when glycemic management was not good (p0.05). Also, none of these inflammatory conditions changed the expression of CAT or SOD1 in a big way ($p > 0.05$).

Amarnath et al. (2011) [24] studied Polyphenols from grape seeds are moved by carboxymethyl chitosan or chitosan hydrochloride in a new nano-antioxidant made by ionic gelation. TEM and FTIR spectroscopy were used to look into the properties of CS-GSPNps. It was determined that the average size of the synthetic CS-GSPNps was 400nm, and that they were not spherical. Cortisol antioxidant activity, lipoprotein oxides (LPO), and CS-GSPNps and grape seed extract (GSE) levels were also measured but also salivary biomarker enzymatic throughout periodontitis (10) patient populations were similar to those in healthy controls (10) .Following therapy with CS-GSPNps as opposed to GSE, statistical analysis revealed significant ($P>0.001$) alterations in the aforementioned parameters. Based on these findings, CS-GSPNps produced with our current approach are potential options for periodontal disease treatment.

Tonguc et al. (2011) [25] examined how smoking affects systemic and local SOD, People with chronic periodontitis have higher levels of GSH-Px, CAT activity, and MDA (CP). The study included 65 CP patients (23 smoking, 23 ex-smokers, and 19 non-smokers) and 20 non-smoking controls with healthy gums and teeth (PH-NS). Samples of serum and gingival tissue were taken after clinical procedures. The use of spectrophotometry to measure the concentrations of SOD, GSH-Px, CAT, and MDA in samples of hemolysates and gingival tissue. Only the disparity between both the CP-FS or PH-NS groups of people with periodontitis was statistically important (P0.01). Groups to periodontitis had gingival tissue with a lot so much MDA than the control condition (P0.01). But in the healthy group, gingival SOD, GSH-Px, or CAT activities were so much higher in the periodontal diseases group (P0.01). The gingiva of the Combination group had higher levels of SOD, GSH-Px, or CAT activity compared to a CP-NS group (P0.01).

Su et al. (2009) [26] aimed to establish if periodontitis is connected with an increase in oxidative damage to DNA, lipids, and proteins, as well as a reduction in the TAC of saliva. 58 individuals with periodontitis and 234 healthy, non-smoking controls had their saliva collected. Characterizing periodontal disease using the CPITN. Metmyoglobin-mediated suppression of ABTS oxidation was used to assess salivary TAC concentrations. I 8-OHdG, 8-epi-PGF2, also

and carbonylated protein have been independently connected negatively to CPITN (P = 0.004, 0.02, and 0.0001); (ii) 8-OHdG, 8-epi-PGF2, but also carbonylated protein were all considerably higher in periodontal patients than controls (P = 0.0003, 0.0001, or 0.0001) and (iii) periodontal patients had significantly more 8-OH Periodontal disease is linked to more oxidatively changed DNA, lipids, as well as proteins in the saliva.

Renvert et al. (2009) [27] investigated analysis of elevated CRP, inflammatory blood markers, or periodontal disease symptoms after treatment with dipyridamole and prednisolone (CRx-102). In this double-blind, only one, placebo-controlled study, patients to 10 pockets but also 5 mm poking and prodding depths were randomly put in either the CRx-102 (n = 28) and placebo (n = 29) groups. Bleeding on probing (BOP), probing depth variations, & inflammatory markers were all measured. Non-invasive mechanical therapy was administered to the participants beginning at day 42, and the trial was terminated at day 49. By day 42, there was a statistically significant difference (P 0.05) between both the two groups on the basis of hs-CRP, IFN-, and IL-6 levels, whereas there was no difference in the other inflammatory biomarkers.Both groups had the same BOP and similar probing depth.

Konopka et al. (2007) [28] investigated indicator of oxidising agent DNA methylation (8-OHdG) and antioxidant capacity level (TAS) on gingiva and peripheral blood during periodontal lesion. 56 people with untreated periodontitis took part in the study. 26 of them had aggressive periodontitis but also 30 had chronic periodontitis (CP). The adults in the control group were all healthy and did not have periodontal disease. A competitive ELISA was used to measure the amount of 8-OHdG. Based just on decrease in the production of ABTSo+ radical cations, a colorimetric method was used to measure TAS. In comparison to the control group, both groups with periodontitis patients had increased levels of 8-OHdG within their gingival blood. The TAS concentrations inside the gum vascular system in CP patients and control subjects were significantly different. The peripheral blood concentration of TAS was significantly reduced in both patient populations versus healthy controls. Excessive levels of TAS were found in the vein and gum blood of everyone who suffered from periodontitis and CP.

Brock et al. (2004) [29] investigated antioxidant capability with periodontal health and illness at the local (saliva & gingival crevicular fluid) or peripheral (plasma or serum) levels Twenty participants of the same gender who did not smoke and age were compared to twenty nonsmoking participants having chronic periodontitis. Whole blood and stimulated and unstimulated saliva were collected after an overnight fast. An improved chemiluminescence technique that has been previously reported was used to quantify total antioxidant capacity (TAOC). Individuals with periodontitis had a considerably decreased content of antioxidants in their gingival crevicular fluid (GCF; p 0.001) compared to healthy controls. Even though periodontitis patients had considerably reduced average TAOC concentrations in both saliva and peripheral blood, only the plasma difference is statistically significant (p 0.05). Healthy participant GCF had significantly more antioxidants than plasma or serum (p 0.001) The results were unaffected by gender-segregated data, and a male bias was seen in all clinical specimens with the exception of GCF.

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METHODOLOGY

The study is being carried out in the Medical College of India in accordance with the ethical guidelines set forth by the World Medical Association for the conduct of medical research involving human subjects. Everyone who took part in the study gave their written consent. Subjects were never under any obligation to participate and could revoke their consent at any time without repercussions.

Study Groups

Overall, 140 individuals suffering from chronic periodontitis, 90 of them were smokers, were enrolled in the study, and 65 seemingly healthy non-smoking male volunteers. Group I consisted of 65 nonsmoking healthy controls (mean age = 40.50 ± 6.15), Group II consisted of 50 nonsmokers with chronic periodontitis (mean age = 42.85 ± 5.45), and Group III consisted of 90 cigarette smokers with persistent periodontitis (mean age = 49.50 ± 6.35). Clinical evaluations for chronic periodontitis in study patients were performed using criteria developed by the Indian Academy of Periodontology. A minimum of twenty teeth were present in each patient, and probing at 30% or more of those teeth indicated a probing depths of 5 mm and a clinical addition of a new of C3 mm. Poor oral hygiene was a contributing factor in the patients' chronic inflammation and bleeding gums upon probing. All respondents said they were in good health and not taking any prescription drugs at the time of the study (including antioxidants, antibiotics, anti-inflammatory meds, etc.) prior to the commencement of the study. Patients with known allergies, those with known diabetes, and alcoholics weren't included. Participants in the study's smoking group were required to have smoked cigarettes regularly for at least 3 years and to smoke an average of five cigarettes daily. The participants in the control group all originated from the same area, shared similar demographics, and were free of dental & systemic health issues as well as cigarette use.

Clinical Measurements

The gingival score (GI) by Loe and also Silness [30], plaque score (PI) by Silness and Loe [31], papillary bleeding score (PBI) by Muhlemann [32], and clinical attachment losses (CAL) score were used to assess periodontal health in all participants. Periodontitis was classified as mild, moderate, or severe based on CAL scores, which were collected from six locations on each tooth (regions around the cheeks, lips, tongue, and gums (masticatory system)). A dentist using a University of India (UNC-15) probe evaluated all clinical parameters. Obtaining Typical Samples Using a single-use syringe, 4 ml of blood was taken from each subject in accordance with established protocols. Serum levels of thioredoxin-coenzyme A (TAOC), red blood cell superoxide dismutase (RBC-SOD), or glutathione peroxidase (Gpx) were all determined using a single millilitre (GPx). An remaining 3 ml of the blood was allowed to settle at room temperature before being centrifuged inside a Serum Separator Clot Activator vacuum centrifuge for 20 minutes to separate the serum (VACUETTE). Preliminary tests necessitated freezing the serum first. Concentrations of vitamin C, malondialdehyde, or C-reactive protein in the blood were measured (CRP). The BIOTRON BTR-830, a semiautomatic analyzer that has been calibrated, was used to measure all biochemical indications (Ranbaxy laboratories, India).

Biochemical Studies

As stated by Benzie FFI and Stain JJ [34], the Ferric Reducing Ability of Plasma (FRAP) test was used to determine the plasma TAOC. As a result of oxidation, Fe_3^{2+}TPTZ (tripyridyltriazine) is reduced to Fe_2^{3+}TPTZ, a blue compound, which is then used to quantify the reaction. Absorbance at 593 nm is measured before and after the addition of ferrous ions to the test liquid, and the relative change in absorbance is used to determine the FRAP value. The amount of redox-coupled superoxide radicals (RCB-SOD) in the cells was assessed using the RANSOD kit. When xanthine and xanthine oxidase are present, superoxide radicals are created, and when they react with 2, 4-idophenyl 3-4 nitrophenol 5-phenyl tetrazolium chloride, they produce the red formazan colour (INT). This reaction's intensity allowed for a calculation of superoxide activity.Under experimental conditions, the rate of INT decline is slowed by 50% for every unit of enzyme activity added. The shift in absorbance was tracked at 505 nm [35]. The RANSEL kit was used to determine GPx levels in the blood. Cumenehydroperoxide oxidises glutathione, and glutaredoxin (GPx) is the catalytic enzyme involved. When oxidised glutathione is combined with NADPH, it is rapidly reduced, while NADPH is simultaneously oxidised to $NADP^+$. It was found that the absorbance drops off sharply at 340 nm [36]. The dinitro-phenyl hydrazine (DNPH) method [37] was used to determine the content of vitamin C (ascorbic acid) in the serum. Ascorbic acid is converted to diketogluonic acid in extremely acidic circumstances, which then combines to 2,4-dinitrophenol dihydrochloride to produce diphenylhydrazone, which then dissociates in concentrated sulfuric to yield a 500 nm-wavelength red complex. The Kei [38] method was used to calculate the MDA content of the blood serum. Serum lipoproteins were precipitated by the addition of trichloro acetic acid. After mixing 0.05 M sulphuric acid & 0.67% thiobarbituric acid (TBA) with 2 M sodium sulphate, lipid peroxide was coupled with TBA via heat. An absorbance reading at 530 nm was taken after the chromogen was extracted in n-butanol. Serum CRP concentrations were measured with the CRP-TURBI & the quantitative latex turbidity technique. Anti-human CRP antibody-coated latex particles agglutinated in the presence of CRP-containing samples. A calibrator with a known CRP concentration [39] can be used to determine how much of an effect the agglutination has on the absorbance (measured at 540 nm) of the test sample.

Statistical Analysis

SPSS for Windows Xp, version 11.5, was used to statistically analyse the clinical parameter and biochemical marker results. For the three groups as a whole, Clinical measures and biochemical indicators' means and standard deviations (SD) were supplied by the study. A percentile to percentile (PP) graph was used to determine the normality of each clinical and biochemical data before any statistics analysis was carried out. Utilizing variance analysis (ANOVA) with India's adjustments just at P 0.05 level of significance, the variations in mean clinical characteristics or biochemical markers between the three study groups were assessed for statistical significance.

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RESULT

Clinical Measurements

Table 1 displays average clinical measurements showing substantially higher clinical parameter levels (P0.001) in group II compared to group I. Clinical indicators for group III also differed significantly (P0.001) from those for groups I & II.

Table 1: Comparing clinical parameters across study groups

Clinical parameter	Mean ± SD			P* value		
	Group I (n= 65)	Group II (n= 50)	Group III (n= 90)	Group I vs Group II	Group I vs Group III	Group II vs Group III
GI	0.8 ± 0.09	2.5 ± 0.7	2.5 ± 0.5	0.001	0.001	0.001
PI	0.6 ± 0.3	2.5 ± 0.8	3.5 ± 0.5	0.001	0.001	0.001
PBI	0.9 ± 0.7	2.7 ± 0.5	2.3 ± 0.5	0.001	0.001	0.001
CAL (mm)	2.0 ± 0.5	7.9 ± 0.9	9.0 ± 1.5	0.001	0.001	0.025

Values are mean ± SD

One-way ANOVA with India's adjustment for multiple group comparisons is used to calculate *p values. P value <0.05, the result can be regarded significant.

Biochemical Studies

Biochemical marker estimates are tabulated and compared between groups statistically in Table 2. Group II markers were found to be distinctive from group I markers (P<0.001). RBC-SOD, MDA, or CRP considerably increased, but TAOC, GPx, and vitamin C decreased dramatically (P 0.001). Additionally, group III considerably differed from group I in terms of biochemical indicators, albeit this difference was not statistically significant when compared to II (except vitamin C).

Table 2: Analysis of biochemical markers between groups

Biochemical markers	Mean ± SD			P* value		
	Group I (n= 65)	Group II (n= 50)	Group III (n= 90)	Group I vs Group II	Group I vs Group III	Group II vs Group III
TAOC (µ M/L)	935.4 ± 69.5	862.4 ± 79.7	812.2 ± 79.9	0.060	0.001	0.082
RBC-SOD (U/g Hb)	311.0± 38.4	535.09 ± 88.7	552.2 ± 70.5	0.001	0.001	0. 998
GPX (U/g Hb)	15.5 ± 1.5	9.1 ± 1.8	8.9 ± 1.2	0.001	0.001	0.345
Vit C (M/I)	40.6 ± 0.0.9	30.5 ± 0.05	23.5 ± 0.005	0.001	0.001	0.001

MDA (Nm/ml)	2.3 ± 0.5	5.2 ± 0.5	4.9 ± 0.5	0.001	0.001	0.565
CRP (mg/l)	2.3 ± 0.5	3.7 ± 0.5	3.7 ± 0.5	0.001	0.001	1.002

Values are mean ± SD

*One-way variance analysis (ANOVA) plus India's adjustment for multiple group comparisons is used to get P values. A p value of 0.05 is required for statistical significance.

CONCLUSION

Patients who suffer from chronic periodontitis have been shown to have more severe clinical periodontal damage, as well as greater levels of systemic oxidative stress and inflammation. According to the results of this study, smokers are also more likely to experience oxidative stress and periodontal inflammation. Chronic periodontitis in smokers can be diagnosed in part by measuring vitamin C levels, a key biochemical marker. The results of this study may provide insight into the detrimental effects of smoking and oral health as well as serve as a clinico-biochemical strategy for more effective treatment of a underlying condition.

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