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Evaluation of Salivary Con centrations of Matrix Metallo proteinase - 8, Tissue Inhibitor of Matrix Metallo proteinases-1, Myelo peroxidase and Nitric Oxide in smokers with Chronic Perio dontitis - A Cross Sectional Analytical Study.

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Abstract

Background: Smoking is the second strongest Modi fiable risk factor for Perio dontal disease. Tissue dest ruction might result from matrix metallo proteinase (MMP)/ tissue inhibitors of MMPs (TIMP) imbalance in the diseased tissues. Myelo peroxidase (MPO) and inducible Nitric-Oxide Synthase (NOS) are stored in and secreted from the primary granules of activated leuko cytes, which may migrate to the site of inflammation and release MMPs. Perio dontal diseases may be associated with systemic diseases. Enhanced circulatory levels of MMPs and their regulators may form a link between the local & systemic conditions. However, no definitive correlation has been established amongst them yet.

Aim of the Study: To evaluate the effects of smoking on salivary levels of MMP-8, TIMP1, MPO and NO in chronic periodontitis.

Objectives of the Study: To devise policy at Govern Ment level for awareness among public regarding adverse effects of smoking for betterment of oral health.

Methodology: 75 males were divided into three groups: Group I- 25 nonsmokers without chronic periodontitis,

Group II - 25 nonsmokers with chronic periodontitis and Group III - 25 smokers with chronic periodontitis. Saliva was collected from all the patients and levels of MMP-8, TIMP-1, MPO and NO were assessed using ELISA kits. ANCOVA and Spearman's partial rank correlation analysis were utilized for statistical analysis.

Results: TIMP-1 values were found to be significantly higher in nonsmoker periodontitis group than smokers with periodontitis ($p< 0.01$). Levels of MPO, MMP-8 and NO were significantly less in smokers with period ontitis group than non-smokers with period ontitis. ($p< 0.01$).

Conclusions: The study showed elevated levels of MMP-8, MPO and NO and reduced levels of TIMP-1 in smokers with periodontitis, when compared to non-smokers with period ontitis. This may be regarded as an indicator of the increased risk for local and systemic in flammation in the patho genesis of period ontal disease, resulting out of oxidative stress caused by cigarette smoke.

Keywords: Chronic periodontitis, Smoking, Matrix Metallo proteinase-8, Tissue Inhibitor of Matrix Metallo proteinases-1, Myeloperoxidase, Nitric Oxide.

Introduction Perio dontal disease is characterized by chronic in flammation along with alveolar bone destruction and connective tissue breakdown.[1]

Smoking is a substantial modifiable risk factor for period ontal disease. Nicotine, a toxic component of tobacco, deteriorates periodontal tissues. Smokers are almost four times more likely to have severe period ontitis than nonsmokers since they harbor a higher prevalence of potential periodontal pathogens, which impairs various aspects of innate and acquired immune responses.[2]

Under normal situations, micro bial virulence factors are balanced by the host response. In period ontitis, this balance is tilted in favor of the micro bial challenge. Smoking is likely to affect the in flammatory response to this microbial challenge by interacting with the host cell. [3]

Periodontal pathogens can lead to an increased release of matrix metallo proteinases (MMPs) by inducing the host cells which can in turn trigger tissue destruction.4 MMP-8, is the major destructive MMP and its excess activity may lead to Perio dontal destruction. MMPs can collectively degrade almost all components of extra cellular matrix and basement membrane.5

It has been shown that imbalance between MMPs and tissue inhibitors of matrix metallo proteinases (TIMPs) can result in tissue destruction during the disease process. TIMPs are expressed by cells that also produce MMPs such as fibroblasts, keratinocytes, mono cytes/ macro phages, and endothelial cells. Among the four TIMPs, 4 TIMP-1 has been demonstrated to be the major inhibitor of MMPs in gingival tissues of patients with periodontal disease.6 TIMPs form classical non-covalent bimolecular complexes with the active form of MMPs and in some instances with the latent MMP precursor as well. TIMPs appears to regulate matrix degradation both by proteinase elimination and by blockage of autolytic MMP activation.[7]

The interaction of TIMPs with MMPs is controlled by a mechanism involving hypochlorous acid (HOCl), a potent oxidant produced by the myeloperoxidase (MPO) system of phagocytes.8 The initial product of the MPO-H2O2 - chloride system is hypo chlorous acid, and sub sequently they form singlet oxygen, chloramines, chlorine, hydroxyl radicals, and ozone. These toxic agents may contribute to periodontal disease patho genesis by attacking the normal tissue when they are released to the outside of the cell. 9 Myelo peroxidase

prevents nitric oxide feedback inhibition and up-regulates the catalytic activity of inducible nitric oxide synthase.[10]

Nitric Oxide (NO) is a secondary messenger acting as a free radical. 11 NO is known for pronounced matrix degradation, especially via suppression of collagen and proteo glycan synthesis through an unregulated metalloproteinase activity, 12 In addition, they have direct effects at bimolecular levels. In higher con centrations, NO can generate reactive nitrogen species (RNS).[11]

Nicotine can generate a systemic inflammatory state via activation of PMNs. Increased neutrophil chemotaxis leads mainly to an increased secretion of MPO & NO from the granulocytes along with elastase & MMPs. Cigarette smoke acts as a major source of free radicals including both reactive oxygen species (ROS) and RNS such as nitric oxide. These reactive substances may enter the blood stream and promote direct oxidative stress, 5 which is known to stimulate nuclear factor kappa B (NF-kB). This leads to inflammatory gene activation and secretion of tumour necrosis factor alpha (TNF-α) and interleukin-8 (IL-8), thereby promoting chronic immune cell recruitment.[13]

Thus, our hypothesis was that the deleterious effects of smoking on the immune system may affect the expression of MMP-8, TIMP-1, MPO and NO, eventually leading to greater periodontal destruction and systemic inflammation. Therefore, this study was under taken to evaluate the salivary levels of MMP-8, TIMP-1, MPO and NO in smokers with periodontitis vs non-smokers with chronic Perio dontitis vis-a-vis Perio dontally healthy controls.

Materials & Methods Before undertaking this study ethical approval was obtained from Ethics Committee of AECS Maruti College of Dental Sciences and Research Centre, Bangalore, India. 75 male patients between the age group 20-75 years were included in the study. Written informed consent was obtained from them after being advised about the nature of the study according to a protocol approved by the Ethics Committee of Maruti College of Dental Sciences & Research Centre, Bengaluru, India (AECS/181 dated 12.12.213). The study was conducted in accordance with the Helsinki Declaration of 1975, as revised in 2013.

Each patient was asked to complete a questionnaire containing details about age, gender, oral hygiene habits, smoking history, medications used and family history. Patients were interviewed for their medical history before the periodontal examination.

Inclusion criteria

According to the classification of American Academy of Period ontology Workshop 1999, patients were diag nosed as “Generalized Chronic Perio dontitis” when attachment loss was ≥5mm at more than 30% of the sites, patients having age ≥35 years and patients who showed radiographic evidence of bone loss. Patients were included in this category only if they had ≥20 functional teeth. “Periodontally Healthy” subjects were those who showed no evidence of periodontal disease (probing depth ≤3mm, no attachment loss, no bleeding on probing, with no detectable radiographic alveolar bone loss). [14]

Assessment of smoking status was performed according to the criteria established by the Centre for the Disease Control and Prevention (CDC).15 “Never” smokers were adults aged 18 years and above, who had smoked less than 100 cigarettes in their lifetime or who had never smoked. “Former” smokers included those who had smoked at least 100 cigarettes in their lifetime, but

will not smoke at the time of interview. "Current" smokers are adults who had smoked at least 100 cigarettes in their lifetime and will be smokers at the time of interview. The "Nonsmoker" category included never smokers and former smokers. The smoker category included current smokers.

Exclusion criteria

Patients were excluded if they (1) suffered from any systemic condition, were alcoholics or were obese (2) had been on antibiotics/ corticosteroids and/ or non-steroidal anti-inflammatory drugs during previous 4 weeks (3) had received professional periodontal therapy during the 6 month period, prior to the study (4) had used mouth rinses containing antimicrobials in the preceding 2 months (5) had any periapical pathology (6) were undergoing orthodontic treatment.

They were separated into three experimental groups: Group I: 25 systemically and periodontally healthy nonsmoking individuals. Group II: 25 patients who were nonsmokers with chronic periodontitis. Group III: 25 patients who were smokers with chronic periodontitis.

Clinical Periodontal Examination

A single, calibrated examiner completed a full mouth assessment of periodontal conditions, except for third molars, using a William's graduated manual probe*. The following parameters were assessed at six sites per tooth (mesio-buccal, mid-buccal, distobuccal, mesio-lingual, mid-lingual, and distolingual).

(1) Plaque Index (PI) 16 (2) bleeding on probing (BOP) 17 (3) probing depth (PD) in mm and (4) clinical attachment level (CAL) in mm. The intra-examiner variability for probing depth (PD) and clinical attachment level (CAL) was 0.32 mm and 0.36 mm respectively.

Saliva collection and processing

Sampling was performed in the morning around 10 AM to avoid a possible variation in the marker con centrations. Whole human saliva was collected using a sterile glass funnel on weighed 10ml sterile poly pro pylene container for 10 minutes. No oral stimuli were permitted for 120 minutes prior to collection to exclude any influence of mastication of food.

Saliva was collected from the seated patients as it flowed into the anterior floor of the mouth over the 10-minute period and drained into a collection tube when necessary. Saliva samples were then frozen immediately at -80˚C until analysis at which point the 8 samples were thawed and cleared by centrifugation at 14000×g for 5 minutes. * Williams's graduated manual probe (Hu Friedy, Chicago, IL).

Biomarker Analysis

Aliquots of each saliva sample were assayed by (Enzyme Linked Immunosorbent Assay) ELISA to determine the levels of MMP-8, TIMP-1, and MPO, according to the manufacturers re commendation. Commercially available ELISA kits† were used to analyze.

The nitric oxide e levels were estimated by assaying the nitrite levels, a stable end product of the nitric oxide metabolism by using Griess reagent. The reaction mixture was then transferred into plastic cuvettes for measurement on a spectrophotometer and readings were taken.

† ELISA test kits were MMP-8 (Boster Biological Technology Co., LTD, California, USA), TIMP-1 (Boster Biological Technology Co., LTD, California, USA) and MPO (Boster Biological Technology Co., LTD, California, USA)

Statistical Method

A sample size of 25 from a population of 75 has a 95% confidence interval with a probability of 0.05 and the sum of square of means equal to 13.32 when the standard deviation for the sample is 0.38.

Therefore, the power of the study was calculated by using the above values and estimated to be 0.96. This infers that a sample size of 25 was adequate to get significant values when all the three groups were compared with each other.

Distribution of clinical and biochemical variables were tested using Shapiro-Wilks test. All biochemical parameters were approximately normally distributed except for age. These numerical variables were log10 transformed and again checked for normality. They were analyzed using One Way Analysis of Variance (ANOVA) with 9 subject groups as factor.

The significance of mean difference between the groups was done by a pair wise comparison using Tukey's Honestly Significant Difference (HSD) test. The pair wise comparison of means were done by One Way Analysis of Co-Variance (ANCOVA) after adjusting age for clinical and biochemical parameters.

Spearman's partial rank correlation analysis was performed to check the association of clinical and biochemical parameters after adjusting for age. A two-sided ($\alpha = 2$) $p < 0.05$ was considered statistically significant. The Statistical Package for Social Sciences software ǂ was used for data processing and data analysis. ‡ Statistical software (IBM SPSS Statistics, Version 21.0. Armonk, NY: IBM Corp.)

Results

Demographic data, clinical and biochemical parameters of the study groups are outlined in Table 1 as mean and standard deviation. One way Analysis of Variance with subject groups as factor showed that all clinical and biochemical parameters were significantly different.

A pair wise comparison using Tukey's HSD showed that except for age, plaque index and probing pocket depth all parameters were significantly different ($p < 0.05$) from patient groups.

The healthy control group exhibited significantly lower values in all clinical parameters. There was no significant difference ($p>0.05$) for age, plaque index and probing pocket depth between smokers with chronic periodontitis (Group III) and non-smokers with chronic periodontitis (Group II).

Table 1–Demographic data, clinical and biochemical parameters of study groups.

Clinical and Bio Chemical Variables	Non Smoker Healthy (n=25)	Non Smoker with Chronic Periodontitis (n=25)	Smoker with Chronic Periodontitis (n=25)	p value
*Age in Years	30.00 (18.00-30.00)	40.00 (30.00-40.00)	40.00 (30.00-48.00)	≤ 0.01
TIMP-1	2.10 (0.41)	0.86 (0.21)	0.41 (0.16)	≤ 0.01
MPO	0.58 (0.15)	1.18 (0.23)	2.61 (0.61)	≤ 0.01
MMP-8	0.30 (0.14)	0.64 (0.25)	1.71 (0.61)	≤ 0.01
NO	0.46 (0.23)	0.85 (0.28)	1.71 (0.39)	≤ 0.01
*PI	0.6 (.30-1.10)	1.80 (1.10-1.80)	1.40 (1.20-1.90)	≤ 0.01
*BOP	14.30 (13.90-18.70)	87.50 (56.20-87.50)	63.20 (56.50-93.20)	≤ 0.01
*PD	1.50 (1.40-1.90)	4.20 (3.90-4.20)	4.20 (4.10-4.50)	≤ 0.01
* CAL	1.50 (1.40-1.90)	5.20 (4.70-5.20)	5.50 (5.40-5.80)	≤ 0.01

Values are Mean ± Standard deviation;

*Values are Median (min-max);

p values are derived using one way analysis of variance

TIMP-1: Tissue inhibitor of matrix metalloproteinase-1 in ng/ml

MMP-8: Matrix metalloproteinase-8 in ng/ml

MPO: Myeloperoxidase in ng/ml

NO: Nitric oxide in ng/ml

PI: Plaque index

BOP: Bleeding on probing

PD: Probing depth in mm

CAL: Clinical attachment loss in mm

The results of Analysis of Co-variance after adjusting with age for clinical and biochemical parameters are tabulated in Table 2.

The pair wise comparison of means showed that except for MMP-8, all clinical and biochemical parameters were statistically significant ($p \leq 0.01$) among all subject groups after adjusting for age. After MMP-8 was adjusted for age, there was no significant difference between healthy (Group I) and nonsmoker with chronic periodontitis (Group II) groups.

TIMP-1 was found to be higher in non-smoker healthy (Group I) followed by non-smoker with chronic periodontitis (Group II) and smokers with chronic periodontitis (Group III). MPO and NO was higher in smokers with chronic periodontitis (Group III) when compared to healthy (Group I) and non-smokers with chronic periodontitis (Group II).

MMP-8 was significantly higher in smokers with chronic periodontitis (Group III) when compared to non-smoker healthy (Group I) and non-smoker with chronic periodontitis (Group II) patients. PI and BOP were higher in non-smoker periodontitis (Group II) followed by smokers with chronic periodontitis (Group III) and non-smoker healthy (Group I) patients.

PD and CAL were higher in smokers with chronic periodontitis (Group III) followed by non-smokers with chronic periodontitis (Group II) and non-smoker healthy (Group I) patients.

Table 2: Analysis of Covariance with age adjusted for clinical and biochemical parameters.

	Non Smoker Healthy (n=25)	Non Smoker with Chronic Periodontitis (n=25)	Smoker with Chronic Periodontitis (n=25)	p value
Biochemical Parameters				
TIMP1	1.589 (0.089)	0.852 (0.055)	0.413 (0.065)	≤ 0.01
MPO	0.620 (0.092)	1.062 (0.057)	2.082 (0.067)	≤ 0.01
MMP8	0.331 (0.154)	0.698 (0.095)	1.617 (0.111)	≤ 0.01
NO	0.473 (0.122)	0.867 (0.075)	1.605 (0.088)	≤ 0.01
Clinical Parameters				
PI	0.894 (0.028)	1.376 (0.017)	1.268 (0.020)	≤ 0.01
BOP	17.153 (1.685)	68.553 (1.040)	58.091 (1.220)	≤ 0.01
PPD	1.772 (0.020)	4.018 (0.012)	4.133 (0.014)	≤ 0.01
CAL	1.772 (0.022)	4.897 (0.013)	5.431 (0.016)	≤ 0.01

Values are Mean ± Standard error of mean;

P values are derived using ANCOVA adjusted for age.

The results of Spearman's partial rank correlation analysis are shown in Table 3. It revealed a significant negative correlation between TIMP-1 and MPO ($r = -0.665$ and $p \leq 0.01$), TIMP-1 and MMP-8 ($r = -0.621$ and $p \leq 0.01$), TIMP-1 and NO ($r = -0.558$ and and $p \leq 0.01$), TIMP-1 and PD ($r = -0.605$ and $p \leq 0.01$) and TIMP-1 and CAL ($r = -0.828$ and $p \leq 0.01$). Except for MMP-8 and PI (p value = 0.04), all values in Table 2 showed a highly significant (p value ≤ 0.01) correlation. A significant positive correlation was noticed between MPO and NO ($r = 0.764$ and $p \leq 0.01$), MPO and PD ($r = 0.612$ and $p \leq 0.01$) and MPO and CAL ($r = 0.854$ and $p \leq 0.01$). NO was positively correlated with MMP-8 ($r = 0.826$ and $p \leq 0.01$) and PD ($r = 0.55$ and $p \leq 0.01$) and CAL ($r = 0.77$ and $p \leq 0.01$).

Table 3 –Correlation analysis with age adjusted for clinical and biochemical parameters of study groups.

Bivariate Correlation	TIMP1	MPO	MMP8	NO	PI	BOP	PPD	CAL
TIMP1	1	-0.665**	-0.612**	-0.558**			-0.605**	-0.828**
MPO	-0.665**	1	0.764	0.725**			0.612**	0.854**
MMP8	-0.612**	0.764**	1	0.826**	-0.237*		0.494**	0.77**
NO	-0.558**	0.725**	0.826**	1			0.55**	0.77**

PI			-0.237*		1	0.818**	0.44**	
BOP					0.818**	1	0.661**	
PPD	-0.605**	0.612**	0.494**	0.55**	0.44**	0.661**	1	0.683**
CAL	-0.828**	0.854**	0.77**	0.77**	`		0.683	1

*Significant at 0.05

**Significant at 0.01

DISCUSSION Chronic periodontitis (CP) is characterized by irreversible alveolar bone loss and connective tissue attachment loss which ultimately results in loss of teeth. 18 Tobacco is the only legitimate drug that kills its emptor when used exactly as intended by Manu facturer's. 19 According to The Tobacco Control Policy Evaluation Project India (the TCP India) Project there are approximately 275 million tobacco users in India. Smoker's harbor potential periodontal pathogens, and thus it can debilitate diversified aspects of innate and acquired immune responses. [20]

Our study evaluated the salivary concentrations of MMP-8, MPO, TIMP-1 and NO in smoker vs non-smoker chronic periodontitis patients as compared to periodontally healthy controls. We also tested to find whether they correlated with each other and with clinical parameters or not. Females were excluded because in our country, not only do very few females smoke regularly but also it would have been difficult to get female volunteers to admit that they smoked. We also wanted to avoid hormone-induced microcirculatory changes.

All clinical parameters were found to be elevated in Groups II and III when compared to Group I ($p<0.01$) This indicates that inflammatory conditions can elevate the clinical parameters. When a comparison was done between Groups II and III, plaque index and bleeding on probing were found to be elevated in non-smokers with period ontitis ($p<0.01$) This is in agreement with previous studies done by Calsina et al.[2] , Feldman et al.[21] and Visvanathan R et al.[22] and Linden and Mullally[23] and Gupta N et.al.[24] The probing depth and clinical attachment levels were elevated in Group III when compared to Group I ($p<0.01$) This can be attributed to the additional inflammatory load in cases of smokers and periodontitis. These results are also in agreement with previous studies Calsina et al.[2], Arno et al.[25] and Haber et al.[26] Our results indicate that smokers, have an increased attachment loss and probing depth.

MMP-8 is a crucial mediator of the irreparable tissue damage associated with periodontitis. [27] It is stored in secondary granules of mature neutrophils as an inactive proenzyme. The first inflammatory cells that arrive at the site of infection are neutrophils. Upon stimulation, they secrete their granular contents which lead to increased levels of pro-inflammatory cytokines i.e., IL-1, TNF alpha, which in turn can lead to an increased release of MMP-8. Similarly bacterial proteinase present in microbial plaque can activate MMP-8 release from PMNs. [28]

Tobacco induced degranu lation, increased pro-in flammatory cytokine burden and alteration in micro bial flora can lead to activation of protein kinase C (PKC), a messenger in the transcriptional regulation of MMP genes and also the secondary messenger, cyclic adenosine monophosphate (cAMP), which can also stimulate MMP expression and cause increased degranulation of neutrophils.28,7 This can lead to increased tissue destruction and aggravated clinical signs of inflammation. MMP-8 levels were found to be highest in Group III (1.62 ng/ml) followed by Group II (0.69 ng/ml) and least in Group I (0.33 ng/ml). A positive correlation was found between MMP-8 and PD ($r= 0.494$, $p= 0.01$) and MMP- 8 and CAL ($r= 0.77$, $p= 0.01$) . These findings suggest that as MMP-8 increases,

inflammation also increases. These results are in agreement with previous studies performed by Knuutinen et al, 29 Betsuyaku et al, 30 Liu et al31 and Gupta N et al.[24]

In our study, TIMP-1 levels were found to be least in smokers with chronic periodontitis (0.413 ng/ml). Non-smokers with chronic periodontitis had a higher level (0.852 ng/ml) and highest levels were found in healthy group (1.589 ng/ml). Thus, as the inflammatory load increases the imbalance between MMPs over TIMPs increases and lower levels of TIMPs are found. A negative correlation was found between TIMP-1 and clinical parameters i.e., PD (r= -0.605, p= 0.01) and CAL (r = -0.828, p= 0.01). Thus, this indicates that reduced levels of TIMP-1 are associated with worsening clinical parameters. These results are in accordance with the studies performed by Soell et al. [32] and Reddy et al. [33]

Myelo peroxidase is an antimicrobial enzyme which is released in the extracellular environment following neutrophil stimulation.[28] MPO was also found to be elevated in Groups II (1.062ng/ml) and III (2.082 ng/ml) when compared to Group I (0.620 ng/ml). Thus, it can be interpreted that in increased inflammation and in smokers, MPO levels are elevated. Similar results were found in a study by Andelid et al.[34] and Raut Elin et al.35 Enhanced serum levels of MPO indicate increased degranulation of specific granules of neutrophils.

In the present study, increase in salivary NO levels were observed in smokers with chronic periodontitis (1.605 ng/ml) and non-smokers with chronic periodontitis (0.867 ng/ml) when compared to healthy group (0.473 ng/ml). Similar results were found in the studies performed by Matejka et al.[36], Lohinai et al.37 and Lappin et al.38 MMP-8 was found to be positively correlated to MPO (r= 0.764, p= 0.01), NO (r= 0.826, p= 0.01) and other clinical parameters. Thus an increase in MMP-8 can lead to an increase in the inflammation.

MMP-8 was also positively correlated with MPO in our study. In an in-vitro study, Saari et al.39 suggested that the hypochlorous acid (HOCl) derived from the myeloperoxidase can activate the direct oxidative pathway for MMP activation. Lint VP et al, 40 suggested that MMP activation involves a cysteine switch mechanism involving the conversion of pro-form of MMP to an active MMP via modification of the cysteine thiol group. The pro-domain of MMP contains a cysteine thiol group which interacts with the Zn+2 ion Winterbourne, 41. For the activation of MMP, this interaction has to be broken. MMP-8 is known to generate reactive oxygen species via breakdown of collagen. According to MPO is maintained in its active form in the presence of excess H2O2, thus potentiating oxidative damage via MPO dependent production of HOCl at the inflammatory site.

Wang Y et al.8 demonstrated in his study that HOCl generated by MPO- H2O2 -chloride system oxidizes the N-terminal cysteine of TIMP-1, thereby controlling the interaction of TIMP-1 with MMP. Thus increased MPO can lead to increased MMP-8 levels and decreased active TIMP-1 levels. Thus a negative correlation of TIMP-1 with MPO (r= -0.665, p= 0.01), and MMP-8 (r= -0.612, p= 0.01) can be justified in our study.

MPO with NO were positively correlated in our study. Eiserich JP et al.[42] and Husam M et al.43 have suggested that the catalytic activity of MPO is biphasic ally 16 modulated by NO. MPO and inducible nitric oxide synthase are both stored in and secreted from the primary granules of activated leukocytes. NO binds to both ferric and ferrous form of MPO, generating a low

spin six coordinate complexes i.e., MPO-Fe (III)·NO and MPOFe (II)·NO respectively thus, inhibiting the catalytic activity of these enzymes by the release of NO from the complex.

Conclusions

The present study demonstrated elevated salivary levels of MMP-8, MPO and NO and reduced levels of TIMP-1 in smokers with chronic periodontitis as compared to non-smokers with chronic periodontitis and non-smokers without chronic periodontitis. This indicates that these analytes maybe linked with the periodontal and smoking status of individuals. Within the limits of the study it can be concluded that inflammation can increase the levels of MMP-8, MPO and NO whereas down regulate TIMP-1.

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