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ASSESSMENT OF THE OXIDATIVE STRESS MARKER SALIVARY MALONDIALDEHYDE IN PERIODONTAL HEALTH AND DISEASE- A CLINICO-BIOCHEMICAL STUDY. AN ORIGINAL RESEARCH

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Abstract
Aim: To evaluate the efficacy of salivary malondialdehyde levels as a diagnostic marker in periodontal disease. **Methodology**: saliva collected from the patients with periodontitis were evaluated for salivary malondialdehyde and also various periodontal parameters were evaluated. **Result:** The findings of this clinical trial suggest a statistically significant direct association between salivary malondialdehyde concentration and chronic periodontal disease. **Conclusion:** A positive correlation between the levels of MDA and all the periodontal parameters was observed.
Keywords: Oxidative stress, Biomarker, Salivary malondialdehyde, Periodontal disease

Introduction: Periodontitis is a term used to describe an inflammatory process, initiated by the plaque biofilm, that is characterized by gingival inflammation, alveolar bone resorption, loss of periodontal attachment to the root surface and adjacent alveolar bone and which ultimately results in tooth loss.[1,2] It is believed that while the primary etiological agent is specific, predominantly gram negative anaerobic or facultative bacteria within the subgingival biofilm [3,4], the majority of periodontal tissue The oxidative

killing mechanism of neutrophils and other phagocytes involve the formation of reactive oxygen species (ROS).[5] A loss of homeostatic balance between proteolytic enzymes (e.g. neutrophil elastase) and their inhibitors (e.g. a1 antitrypsin) and reactive oxygen species (ROS) and the antioxidant defense systems that protect and repair vital tissue, cell, and molecular components is believed to be responsible. The basis for such dysregulation is in part genetic (38–82%) (270) and in part the result of environmental factors (e.g. smoking) [6]. ROS is a collective term which includes oxygen derived free radicals (ODFR), such as the superoxide radical (O2 −), hydroxyl radical (OH) and nitric oxide radical (NO) species, and non-radical derivatives of oxygen, such as hydrogen peroxide (H2O2) and hypochlorous acid (HOCl). The presence of one or more unpaired electrons in the outer orbitals of ODFR makes such species, especially the OH species, extremely reactive in nature (Halliwell et al, 1992).[7] Oxidative stress has been implicated as a major contributor in over 100 disorders and more recently periodontitis.[2] The pathological events leading to the destruction of periodontium during inflammation has been related to oxidative stress. It occurs as a consequence of imbalance between the formation of free oxygen radicals and inactivation of these species by antioxidant defence system, and is capable of causing damage to various cellular and extracellular constituents.[1] Free radicals are a family of highly reactive and diverse species, capable of extracting electrons and thereby oxidizing a variety of bio molecules vital to cell and tissue function, which not only includes oxygen free radicals, but also nitrogen and chlorine species.[8,9] The deleterious effects of increased oxidative stress are termed oxidative damage that generally appear after exposure to a relatively high concentration of reactive oxygen species (ROS) and/or a decrease in antioxidant (AO) defence system against ROS.[2] ROS are produced continuously in living cells as byproducts of normal metabolism. They are highly toxic, not only to the internalized microbial agent, but also to the extracellular structure and can induce lipid peroxidations (LPO) having effects on cells.[3] Redundant production of LPO can result in oxidative stress and consequently, damage to cell integrity. Because LPO results from oxidative stress, numerous markers have been used to monitor this process.[10] Malondialdehyde (MDA) is the principal and most studied product of polyunsaturated fatty acid peroxidation that can indicate the increase of oxidative stress. It is a low molecular weight end product of lipid peroxidation which is highly reactive organic compound capable of causing toxic stress in cells and form covalent protein adducts referred to as advanced lipoxidation glycation end products (AGE).[4] It is typically not observed in pure form and has a formula of $CH2(CHO)^2$. MDA is the best known specific thiobarbituric reacting substance. They are known to contribute to the numerous pathogenic processes. Recently it has been indicated that periodontitis is an oxidative stress state.[11,12] Tissue damage is due to the consequence of the phagocyte mobilization and activation. Oxidation of important biomolecules or indirectly activation of the transcription factors involved in redox reactions lead to a distant reaction of the proinflammatory genes possibly associated with systemic response[13]. This could possibly be the link in between oxidative mechanism and periodontitis. Saliva being non-invasive, easy to collect, convenient approach that is bearable to the patient can be used to assess MDA levels. A major challenge in clinical periodontics is to find a reliable

molecular marker of periodontal tissue destruction with high sensitivity, specificity and utility. Hence the aim of this study is to assess the correlation between the level of malondialdehyde in saliva and periodontal status.

Methodology: In this study, 60 participants, aged between 35-70 years were examined from the outpatient Department of Periodontics of A.J. Institute of Dental Sciences. An informed consent was taken from all these subjects. A brief case history was recorded and the subjects were divided into 2 groups (n=30). The following criteria were considered for the selection of subjects:

Inclusion criteria

1. Individuals with clinically healthy periodontium were considered for this study as a control group.
2. Age group between 35 to 70 years.
3. Patients who exhibited generalised form of periodontal destruction with clinical evidence of bleeding on probing and periodontal pockets measuring ≥5 mm in more than 30% of the sites.
4. Subjects with minimum complement of 20 teeth.

Exclusion criteria

1. Patients who had undergone any periodontal, surgical or nonsurgical therapy for the past 6 months.
2. Patients who had received any chemotherapeutic mouth rinse or oral irrigation during the past 6 months.
3. Patients who had received antibiotics and anti-inflammatory drugs in the past 4-6 weeks.
4. Patients with a history of underlying systemic diseases and conditions.
5. Patients with habit of smoking, tobacco chewing and alcohol consumption.
6. Pregnant and lactating women and those using hormonal contraceptives.

Ethical consent Permission for this study was obtained from the Ethical Committee of A.J. Institute of medical Sciences and Research Centre. Examiner Calibration Study was carried out by a single examiner throughout the study period.

Methodology: For the study 60 adult subjects were selected from Outpatient Department, A.J. Institute of Dental Sciences, Mangalore. The study sample consisted of 60 subjects (both male and female) and with age ranging from 35-70 years. Before starting the study, the health of the subjects was ascertained by using detailed medical history and clinical examination following the inclusion, exclusion criteria mentioned above. Subjects were given informed consent forms prior to clinical examination. All the periodontal disease measurements were performed in 4 quadrants using a Williams Graduated Periodontal Probe. Periodontal status was assessed by recording full mouth bleeding on probing and measuring probing pocket depth at 4 sites (Mesio Buccal, Midbuccal, Distobuccal, Midlingual) per

tooth. Subjects were divided into 2 groups (n=25) based on gingival and periodontal condition. The "Power and Sample Size Calculations: A Review and Computer Program" was used for sample size calculation. A sample size formula was derived for this independent, case control study:

$$n = \frac{2(Z\alpha + Z\beta)^2 \sigma^2}{\delta 2}$$

$Z\alpha = 1.96$ (95% Confidence level)

$Z\beta = 1.28$ (90% power)

σ = combined standard deviation

δ = mean deviation

Clinical parameters: All the patients were assessed on the basis of gingival and periodontal status. Clinical examination was carried out using a Williams Graduated Periodontal Probe.

Clinical parameters recorded were: -

Plaque index [Silness and Loe (1964)]

Gingival index [Loe and Silness (1963)]

Probing pocket depth (PD)

Clinical attachment level (CAL)

Patients were grouped as healthy and chronic periodontitis as follows-

Healthy controls	Sulcus bleeding index is 0-1 and gingival sulcus depth is ≤ 3mm.
Chronic periodontitis	sulcular bleeding index is 2-5 Probing periodontal pockets ≥ 5mm. ≥ 30% affected teeth.

Collection of saliva: Sample collection was made at the standardized time according to the diurnal cycle between 8 AM to 10 AM. Subjects were instructed not to eat or rinse within 60 minutes prior to sample collection. Around 2 ml of whole unstimulated saliva was collected simply by drooling into a sterilized vial with the forward tilted head or by allowing the saliva to accumulate in the mouth and then expectorate into a vial. The resulting saliva was centrifuged immediately at 3000 rpm for 10 min. The clear supernatant was collected and stored in aliquots at -20°C until the further analyses were performed.

Determination of malondialdehyde: The Thiobarbituric Acid Assay Malondialdehyde, formed from the breakdown of polyunsaturated fatty acids, serves as a convenient index for determining the extent of the peroxidation reaction. Malondialdehyde has been identified as the product of lipid peroxidation that reacts with thiobarbituric acid to give a red species absorbing at 535 nm. Reagent Stock TCA-TBA-HCI reagent: 15% w/v trichloroacetic acid; 0.375% w/v thiobarbituric acid; 0.25 N hydrochloric acid. This solution may be mildly heated to assist in the dissolution of the thiobarbituric acid. Procedure:

Combine 1.0 ml of saliva with 2.0 ml of TCA-TBA-HCI and mix thoroughly. The solution is heated for 15 min in a boiling water bath. After cooling, the flocculent precipitate is removed by centrifugation at 1000 g for 10 min. The absorbance of the sample is determined at 535 nm against a blank that contains all the reagents minus saliva. The malondialdehyde concentration of the sample can be calculated using a molar extinction coefficient of 1.56 × 105 and expressed in mmol per ml.

Statistical analysis: The statistical analysis was performed using SPSS version 23.0 and statistical significance was defined as $p \leq 0.05$. The descriptive analysis was performed for the healthy and chronic periodontitis patients. The Pearson's correlation between the clinical parameters like Plaque Index (PI), Gingival Index (GI), Pocket Depth (PD) Loss of Attachment (LOA) with salivary malondialdehyde was also performed. The Student's unpaired 't' test was done to compare the various clinical parameters with salivary malondialdehyde.

Results: The present clinico-biochemical study was carried out to estimate the salivary malondialdehyde level in periodontal health and disease. There were total of 60 subjects, in the age group of 35-70 years who participated in the study. Depending on the gingival and periodontal status, subjects were categorized into 2 groups of 30 subjects each. Two groups being: Group A- Healthy controls and Group B- Chronic periodontitis. Plaque index, gingival index, probing pocket depth, loss of attachment and BMI were assessed in all 60 subjects. The variations in gingival and periodontal status of both the groups were determined. Salivary Malondialdehyde in both the groups were also evaluated. The statistical analysis was performed using SPSS version 23.0 and statistical significance was defined as p value ≤ 0.05. The findings obtained were systematically tabulated and subjected to statistical analysis using Pearson's correlation and Student's unpaired 't' test. A correlation between various clinical parameters were analysed. The comparison of gingival index, plaque index, pocket depth and loss of attachment with salivary malondialdehyde were done using Student's unpaired t-test. Profile of Sample Distribution of healthy and Chronic periodontitis cases in the sample Patients aged 35-70 years were randomly selected to participate in the study. A total of 60 patients were selected and divided into Group A- Healthy and Group B-Chronic Periodontitis Group. (Table 1)

Table 1-Distribution of the Sample

	Frequency	Percent
Group A-Healthy	30	50.0
Group B-Chronic Periodontitis	30	50.0

Distribution of Male and Female in the Sample: Among the 30 patients in Group A, 12 females and 18 males participated in the study. In the healthy group 40 percent of the cases was females and 60 percent was males. (Table 2)

Table 2- Distribution of gender in Group A

Healthy	Frequency	Percent
Female	12	40.0
Male	18	60.0
Total	30	100.0

Among the 30 patients in Group B, 13 females and 17 males participated in the study. In the healthy group 43.3 percent of the cases was females and 56.7 percent was males. (Table 3)

Table 3- Distribution of gender in Group B

Chronic periodontitis	Frequency	Percent
Female	13	43.3
Male	17	56.7
Total	30	100.0

Distribution of Healthy and chronic periodontitis cases in different age ranges. The age wise distribution of cases in Group A showed that between the range of 36-40 years, 41-45 years, 46-50 years, 51-55 years and 56-60 years, a frequency of 8,10,6,3,3 respectively and a percentage of 26.7%, 33.3%, 20%, 10%, 10% respectively was observed. The age wise distribution of cases in Group A showed that between the range of 36-40 years, 41-45 years, 46-50 years, 51-55 years and 56-60 years, a frequency of 4,9,11,4,2 respectively and a percentage of 13.3%, 30%, 36.7%, 13.3%, 6.7% respectively was observed. (Table 4)

Table 4- Distribution of Healthy and chronic periodontitis cases in different age ranges

Age range	Healthy		Chronic periodontitis	
	Frequency	Percent	Frequency	Percent
36-40	8	26.7	4	13.3
41-45	10	33.3	9	30.0
46-50	6	20.0	11	36.7
51-55	3	10.0	4	13.3
56-60	3	10.0	2	6.7
Total	30	100.0	30	100.0

Descriptive statistics: In descriptive analysis of the healthy sample, the body mass index, Plaque index, gingival index, loss of attachment and salivary malondialdehyde was analysed. The minimum, maximum, mean and standard deviation was tested.

Body mass index: The body mass index had a minimum of 20.30, maximum of 30.60, mean of 27.1633 and a standard deviation of 2.45995 in the healthy group whereas the Chronic periodontitis group had

a minimum of 20.22, maximum of 32.40, mean of 27.1973 and a standard deviation of 27.1973. (Table 5)

Table 5- Mean Body Mass Index for Group A and Group B

Body Mass Index	Healthy				Chronic Periodontitis			
	Minimum	Maximum	Mean	Std. Deviation	Minimum	Maximum	Mean	Std. Deviation
	20.30	30.60	27.1633	2.45995	20.22	32.40	27.1973	27.1973

Plaque index: The plaque index had a minimum of 0.70, maximum of 2.20, mean of 1.09213 and a standard deviation of 0.499928 and a minimum of 20.22, maximum of 32.40, mean of 27.193 and a standard deviation of 27.1973 in the healthy and chronic periodontitis group respectively. (Table 6)

Table 6- Mean Plaque Index for Group A and Group B

Plaque Index	Healthy				Chronic Periodontitis			
	Minimum	Maximum	Mean	Std. Deviation	Minimum	Maximum	Mean	Std. Deviation
	.070	2.200	1.09213	.499928	20.22	32.40	27.1973	27.1973

Gingival index: Gingival index showed, 0.02, 0.63, 0.2033and 0.15305 minimum, maximum, mean and standard deviation respectively in the healthy group and 0.75, 2.78, 1.5993, 1.5993 minimum, maximum, mean and standard deviation respectively in the Chronic periodontitis group. (Table 7)

Table 7- Mean Gingival Index for Group A and Group B

Gingival Index	Healthy				Chronic Periodontitis			
	Minimum	Maximum	Mean	Std. Deviation	Minimum	Maximum	Mean	Std. Deviation
	.02	.63	.2033	.15305	.75	2.78	1.5993	1.5993

Pocket depth: The pocket depth's minimum, maximum, mean and standard deviation was 1.830, 3.200,2.16457, 0.283714 respectively in the healthy group and 2.600, 4.12, 3.33567, 3.33567 respectively in the chronic periodontitis group. (Table-8)

Table 8- Mean Pocket Depth for Group A and Group B

	Healthy	Chronic Periodontitis

Pocket depth	Minimum	Maximum	Mean	Std. Deviation	Minimum	Maximum	Mean	Std. Deviation
	2.600	4.120	3.335 67	3.33567	.62	5.60	3.272 0	3.2720

Loss of attachment: The minimum, maximum, mean and standard deviation for loss of attachment was 0.00,0.60, 0.0577,0.13343 respectively for healthy group and 0.62, 5.60, 3.2720, 3.2720 respectively for chronic periodontitis group. (Table-9)

Table 9- Mean Loss of Attachment for Group A and Group B

Loss of Attachment	Healthy				Chronic Periodontitis			
	Minimum	Maximum	Mean	Std. Deviation	Minimum	Maximum	Mean	Std. Deviation
	.00	.60	.0577	.13343	.62	5.60	3.2720	3.2720

SALIVARY MALONDIALDEHYDE The salivary malondialdehyde's minimum, maximum, mean and standard deviation was 0.0200, 5.7720, 3.279927 and 1.6814262 respectively in the healthy group and 7.7320, 15.3970, 10.769633 and 1.7916411 respectively in the chronic periodontitis group. (Table 10)

Table 10-Mean Salivary Malondialdehyde (mmol/ml) of group A and group B

Salivary Malondial dehyde	Healthy				Chronic Periodontitis			
	Minimum	Maximum	Mean	Std. Deviation	Minimum	Maximum	Mean	Std. Deviation
	.0200	5.7720	3.279 927	1.6814262	7.7320	15.3970	0.76 9633	1.7916411

Correlation between different parameters: The correlation between salivary malondialdehyde and age, gender and BMI were performed using Pearson's correlation. Correlation between Salivary Malondialdehyde and Age The correlation between salivary malondialdehyde and age gave a score of 0.079 and a p value of 0.550 which was statistically non-significant. (Table 11)

Table 11-Pearson's Correlation between Salivary Malondialdehyde and Age

		AGE
SALIVARY MALONDIALDEHYDE	Pearson Correlation	.079
	Sig. (2-tailed)	.550 NS

P< 0.05- sig- significance

P< 0.01- HS- highly significance

P< 0.001- VHS- very highly significance

P< 0.05- NS - non-significant

Correlation between Salivary Malondialdehyde and Gender.

The Pearson's correlation between salivary malondialdehyde and gender had a score of 0.016 and the p value was 0.903 which was statistically non-significant. (Table-12)

Table 12-Pearson's Correlation between Salivary Malondialdehyde and Age

		Gender
SALIVARY MALONDIALDEHYDE	Pearson Correlation	.016
	Sig. (2-tailed)	.903 NS

Correlation between Body Mass Index and Salivary Malondialdehyde The Pearson's correlation between salivary malondialdehyde and Body mass index was 0.077 and the p value was 0.557 which was statistically non-significant. (Table-13)

Table 13-Pearson's Correlation between Salivary Malondialdehyde and Body Mass Index.

		Gender
SALIVARY MALONDIALDEHYDE	Pearson Correlation	.077
	Sig. (2-tailed)	.557 NS

Correlation between Salivary Malondialdehyde and plaque index, gingival index, pocket depth, loss of attachment. The Pearson's correlation of salivary malondialdehyde and plaque index, gingival index, pocket depth, loss of attachment was 0.561, 0.849, 0.809 and 0.771 respectively. With a p value of 0.00, they were all statistically highly significant. (Table 14) (Graphs-9,10,11,12)

Table 14-Pearson's Correlation between Salivary Malondialdehyde and plaque index, gingival index, pocket depth, loss of attachment.

		SALIVARY MALONDIALDEHYDE
Plaque index	Pearson Correlation	.561**
	Sig. (2-tailed)	.000(HS)
Gingival index	Pearson Correlation	.849**
	Sig. (2-tailed)	.000(HS)
Pocket depth	Pearson Correlation	.809**
	Sig. (2-tailed)	.000(HS)
Loss of attachment	Pearson Correlation	.771**
	Sig. (2-tailed)	.000(HS)

Student's Independent 't' Test

The Student's Independent t test was performed to compare the Plaque index, Gingival index, Loss of attachment and Probing pocket depth in both the groups.

Comparison of the Plaque index in Group A and Group B The t value for the plaque index between Group A and Group B was -6.108 and the p value was 0.00 which implied it was statistically highly significant. (Table 15)

Table 15- Comparison of the Plaque index in Group A and Group B

Plaque index	t value	P	Mean Difference	95% Confidence Interval of the Difference	
				Lower	Upper
	6.108	0.000	-.825200	-1.095655	-.554745

Comparison of the Gingival index in Group A and Group B The t value for the gingival index between Group A and Group B was -15.241 and the p value was 0.00 hence it was statistically highly significant. (Table 16)

Table 16- Comparison of the Gingival index in Group A and Group B

gingival index	t value	P	Mean Difference	95% Confidence Interval of the Difference	
				Lower	Upper
	-15.241	0.000	-1.39600	-1.57934	-1.21266

Comparison of the Probing Pocket depth in Group A and Group B The t value for the pocket depth for Group A and Group B was -14.039 and the p value was 0.00 which implied it was statistically highly significant. (Table-17)

Table 17- Comparison of the Probing Pocket Depth in Group A and Group B

Pocket depth	t value	P	Mean Difference	95% Confidence Interval of the Difference	
				Lower	Upper
	-14.039	0.000	-1.171100	-1.338084	-1.00411

Comparison of the Loss of Attachment in Group A and Group B The t value for loss of attachment for Group A and Group B was -12.552 and the p value was 0.00 hence it was statistically highly significant. (Table 18)

Table 18- Comparison of the Loss of Attachment in Group A and Group B

Loss of attachment	t value	P	Mean Difference	95% Confidence Interval of the Difference	
				Lower	Upper
	-12.552	0.000	-3.21433	-3.72695	-2.70172

Comparison of the Salivary malondialdehyde in Group A and Group B The t value for salivary malondialdehyde between Group A and Group B was -16.696 and the p value was 0.00 which implied it was statistically highly significant. (Table 19)

Table 19- Comparison of the Salivary Malondialdehyde in Group A and Group B

Salivary malondialdehyde	t value	P	Mean Difference	95% Confidence Interval of the Difference	
				Lower	Upper
	-16.696	0.000	-7.4897067	-8.38767	-6.59174

Discussion: Periodontitis is an acute or chronic inflammatory process, initiated by the plaque biofilm, which causes loss of attachment of periodontal tissue to the root surface and adjacent alveolar bone and which ultimately results in tooth loss [14]. The abnormal host response to subgingival biofilm leads to the progressive destruction of the tissues. An imbalance in the homeostasis is observed due to proteolytic enzymes & their inhibitors, reactive oxygen species (ROS) and the antioxidant defence systems and pro inflammatory and anti-inflammatory cytokines.

A potential mechanism in periodontal tissue breakdown could be alterations in the oxidative stress-mediated inflammatory pathways. Oxidative stress represents an increase in the production of oxidants and/or a decrease in antioxidants, i.e. an oxidant/antioxidant imbalance. Oxygen is an essential element of aerobic life, but when partially reduced, it generates reactive oxygen species (ROS) [15]. An unbalance between radical and non-radical ROS causes peroxidation of lipid membranes. Large amounts of pro-oxidants are produced in the prolonged inflammatory responses, which occur in the context of gingivitis and periodontitis[16].

Saliva has been used widely as a diagnostic tool for periodontal diseases as it measures the concentration of lipid peroxidation products and enabling the extent of tissue damage in patients[17]. We can assess the extent of tissue damage in a diseased patient by measuring the concentration of lipid peroxidation products.

Malondialdehyde (MDA) is one of many low molecular weight end-products of lipid peroxidation (LPO) and is the most often measured as an index of peroxidation[18]. It is the principal and most established product of PUFA peroxidation showed to increase following oxidative stress. An increase in the levels of MDA can indicate oxidative stress and give us an idea about the progression of the disease.

A study conducted by **Shetty S et al** [19] to analyse the levels of salivary malondialdehyde in healthy and patients with chronic periodontitis found an association between the MDA levels and disease progression[46]. Therefore, in the present study, an attempt was made to investigate the estimation and correlation of salivary malondialdehyde levels in periodontal health and disease. A total of 60 subjects in the age group of 35-70 years were included in the study, out of which 30 had clinically healthy gingiva and remaining 30 had chronic periodontitis. Patients were classified as having chronic periodontitis if they clinically showed pocket depth of ≥5mm in > 30% of dentition.

In the present study, we observed that the salivary malondialdehyde levels increased significantly in patients with chronic periodontitis when compared to healthy group. These results were similar to the findings by **Shetty S et al** [19].

A positive correlation between the periodontal parameters like bleeding on probing, plaque index, gingival index, pocket depth and loss of attachment was found. These were in accordance with the studies conducted by **Khalili J et al, Ahmadi F et al** [20,21] which indicated an increase in the periodontal parameters leading to an increase in the salivary malondialdehyde levels, suggesting the progression of the disease [26,51]. An increase in the severity of the disease was observed with an increase in the levels of salivary malondialdehyde.

In a study conducted by **Miricescu D et al** [22] found an increase in the oxidative stress associated alveolar bone loss biomarkers. An increase in attachment loss leading to an increase in levels of salivary malondialdehyde was comparable to the results in the study. When compared between the gender, there was no difference observed in the levels of the salivary malondialdehyde between males and females which was similar to the study conducted by **Ahmadi F et al** [21]. There was statistically no significant difference between the levels of salivary malondialdehyde in patients of different age groups.

Akalin FA et al [23] found that saliva and GCF MDA levels were significantly higher in patients with chronic periodontitis whereas there was no difference found in the serum MDA levels between healthy and control group. This suggested that saliva can be a better indicator of variations of the levels of MDA. Similar results were obtained in a study conducted by **Tripathi V et al** [24].

In this study the salivary MDA levels seemed to directly co-related to the various clinical parameters of periodontal disease and a statistically significant difference was seen in various parameters with salivary MDA. **Tamaki et al** [25] compared the salivary MDA before and after the non-surgical periodontal therapy in patients with chronic periodontitis. A significant reduction in the reactive oxygen metabolites was observed with an improvement in the clinical parameters.

In an experimental study by **Yagan et al** [26] to observe the effect of Low-Dose Doxycycline on Serum Oxidative Status, Gingival Antioxidant Levels, and alveolar bone loss in rats, they found that alveolar bone loss was significantly higher in the experimental periodontitis group compared with the experimental periodontitis and LDD and control group. Also, doxycycline exhibited the most prominent inhibition on gingival tissue levels of MDA and antioxidant enzymes as the doxycycline pre-treatment significantly reduced the increase in lipid peroxidation.

According to the results of this study, a significant difference in the levels of salivary malondialdehyde is found between chronic periodontitis and control. Salivary malondialdehyde appeared to be positively correlated in both the groups, which suggests that the increase in the lipid peroxidation due to inflammatory reactions is seen in periodontal disease.

Studies conducted so far have all confirmed an increase in the salivary MDA levels in periodontal disease.

Taking to considerations all the parameters and because of the cross-sectional design of the present study, a clear cause-effect relationship between salivary malondialdehyde and periodontal disease cannot be confirmed. Larger and more randomized controlled clinical trials are required to

substantiate the correlation between periodontal disease and salivary malondialdehyde concentration in saliva and also its role as a diagnostic marker in periodontal disease.

Conclusion: The findings of this clinical trial suggest a statistically significant direct association between salivary malondialdehyde concentration and chronic periodontal disease. A positive correlation between the levels of MDA and all the periodontal parameters was observed. There was a significant increase in salivary malondialdehyde concentration with an increase in loss of attachment. Therefore, salivary malondialdehyde levels can be used to monitor the severity of periodontal disease. In order to explore the actual cause to effect relationship between periodontal disease and malondialdehyde concentration, longitudinal evaluations with a larger population would be necessary.

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