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Evaluation of the diagnostic efficacy of salivary malondialdehyde among smokers and nonsmokers with periodontal disease: A case-control study

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Abstract:

Reactive oxygen species (ROS) are molecules that are produced by a variety of metabolic processes that can lead to oxidative stress and subsequent direct or indirect tissue damage, are linked to the pathogenesis of the majority of inflammatory processes and result in periodontal disorders. Lipid peroxidation product malondialdehyde (MDA) caused by ROS can lead to tissue injury. The aim of this study was to assess and compare the concentrations of malondialdehyde (MDA) in periodontal health and periodontal disease in nonsmokers and smokers, and to establish its usefulness as diagnostic indicators for periodontal disease.125 male patients were categorized as control: 25 healthy nonsmokers,25 nonsmokers with gingivitis,25 smokers with gingivitis, 25 nonsmokers with periodontitis and 25 smokers with periodontitis. For at least 10 minutes, 2ml of unstimulated saliva was collected. Periodontal clinical parameters (PI, BOP, PPD and CAL) were recorded to assess each subject's periodontal health. An enzyme-linked immunosorbent assay measured salivary MDA levels. All groups had higher levels of salivary MDA ($P < 0.05$) when compared to the healthy controls, except for the nonsmoker periodontitis group was not statistically different from the control group; however, smoker groups had higher MDA levels than nonsmoker groups. With the limitations of this case-control study, it may be suggested that the oxidative stress biomarker MDA was higher in periodontal diseases associated with risk factors such as smoking and this biomarker has the potential to be employed as a diagnostic biomarker for periodontal diseases.

Keywords: periodontal disease, malondialdehyde, reactive oxygen species, smoking, tobacco use.

1. Introduction:

The biomedical research aims to use experimental findings in therapeutic settings. The presence of microbial infection in susceptible individuals is the hallmark of periodontal illnesses, which lead to the degradation of the periodontal tissues that support teeth and the possibility of tooth loss. Gingivitis and periodontitis are the two most commonly observed manifestations of these disorders (Ball and Darby, 2022).

Gingivitis, the mildest form of periodontal illness, arises as a consequence of the accumulation of bacterial biofilm, generally referred to as dental plaque, in close proximity to the gingiva. Significantly, it is important to note that this state has the potential to be reversed (Pihlstrom et al., 2005). Periodontitis is a persistent inflammatory condition that causes periodontal tissues to deteriorate due to host cell secretion of inflammatory cytokines and reactive oxygen species (ROS) overproduction in vulnerable individuals (Cekici et al., 2014).

The concept of "reactive oxygen species" (ROS) comprises a diverse range of molecular oxygen derivatives that occur spontaneously in aerobic organisms. The occurrence of elevated levels of diversity (ROS) leads to molecular damage, commonly known as 'oxidative stress' (Sies and Jones, 2020).

Oxidative stress can be characterized as a state in which there is an equilibrium between an overabundance of reactive oxygen species (ROS) and a concurrent insufficiency of antioxidants (Miricescu et al., 2014). However, the duration of (ROS) is notably short, posing a significant challenge in detecting them. Therefore, the analysis of breakdown products formed from (ROS) and the evaluation of both enzymatic and non-enzymatic antioxidants are ideal candidates for investigating the consequences of oxidative stress-related products in the pathological development of Periodontitis (Chen et al., 2019).

The overproduction of free radicals can lead to increased levels of oxidative stress, which in turn causes oxidative damage to biological components, hence contributing to the onset of numerous chronic diseases (Madhloom and Diajil, 2020).

In relation to periodontitis, the consumption of tobacco has the capacity to increase the activity of polymorphonuclear neutrophils, leading to the emergence of a hyperactive condition in these cells. Consequently, this phenomenon gives rise to an increased secretion of proinflammatory cytokines and an overabundance of (ROS) via a mechanism referred to as respiratory burst. Hence, the destructive impact on gingival tissue is attributed to the oxidative stress induced by these mechanisms (Palmer et al., 2005, Johannsen et al., 2014, Nociti Jr et al., 2015, Sczepanik et al., 2020). There exists a positive correlation between the increase in smoking frequency and the concomitant increase in the severity of periodontal disease (Humadi, 2016, Mohammed et al., 2023).

MDA, an extensively studied lipid peroxidation product, serves as a reliable indicator for assessing oxidative stress. It has gained recognition as a well-established tool in the field of periodontitis research (Ahmadi-Motamayel et al., 2017). The quantity of MDA in physiological fluids may be a good predictor of the severity of oxidative damage to human cells, according to a large body of empirical research (Tsikas, 2017). Existing research shows that LPO products like MDA are generated during periodontal disease and contribute to the progression of periodontal tissue inflammation and damage

Another study that showed that patients with periodontitis and smokers have higher levels of lipid peroxidation in their saliva as compared to healthy controls further supported the significance of lipid peroxidation in saliva (Guentsch et al., 2008). Finding an accurate molecular indicator of periodontal tissue deterioration with good sensitivity, specificity, and utility is a significant challenge in clinical periodontics (Braz-Silva et al., 2019).

A biomarker is an attribute that can be quantified to show whether biological systems are working normally or abnormally, as well as how they are responding to treatments or outside influences. Biomarkers have demonstrated significant promise in the diagnosis and monitoring of several illnesses (Maher Abdulazeez Nsaif, 2023, Yosuf, 2019, Abdul-Mounther et al., 2017).

The aim of this study is to assess the diagnostic efficacy of salivary levels of MDA in patients with periodontal disorders (gingivitis and periodontitis) in comparison to those who are periodontally healthy, taking into account the patients' smoking habits.

2. Materials and methods:

2.1 Study design:

In this case-control study (125) men are selected. From March to June 2023, this study was undertaken in the teaching clinics of the periodontics department at the University of Anbar College of Dentistry. Following ethical clearance from the University of Baghdad College of Dentistry Ethics Committee (Ref.737, 1/12/2022, Project #737622). The study is carried out in accordance with the World Medical Association's (WMA) Helsinki Declaration's ethical guidelines. All participants signed an informed consent form after obtaining study information.

After recruitment, participants are grouped as healthy periodontium (Control), nonsmokers gingivitis, smokers gingivitis, nonsmokers periodontitis, and smokers periodontitis. The control criteria encompassed a BOP (bleeding on probing) value of less than 10%, a PPD (probing pocket

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depth) measurement of not more than 3 mm, and the presence of an intact periodontium without any attachment loss (Chapple et al., 2018). Diagnostic criteria for gingivitis include BOP > 10%, PPD ≤ 3 mm, and intact periodontium (Chapple et al., 2018). Individuals diagnosed with periodontitis should exhibit a generalized form (30% of teeth involved) and unstable status (PPD 5mm or PPD 4mm with bleeding on probing [BOP]) (Tonetti et al., 2018).

2.2 Inclusion and exclusion criteria:

The study includes willing subjects with more than 20 teeth and no history of systemic illness. Current smokers who had been smoking for more than three years with at least 10 cigarettes per day were included in the smokers group (Santos et al., 2015). On the other hand, the study excludes former smokers and those who used tobacco products in another form in addition to cigarettes.

Conditions that exclude subjects from the study include 1) Systemic illness; 2) recent use of antimicrobials, antioxidants, anti-inflammatory drugs, or other treatments that may have an impact on periodontal health; 3) Alcoholics; 4) People who have recently undergone periodontal therapy; and 5) People who frequently use antimicrobial mouthwash.

2.3 Clinical examination:

The individuals' periodontal charts have comprehensive data, encompassing measurements of plaque index (PI), bleeding on probing (BOP), probing pocket depth (PPD), and clinical attachment loss (CAL). The clinical periodontal parameters, with the exception of wisdom teeth, are examined at six sites per tooth. However, for plaque scores, only four surfaces are employed by a calibrated periodontist using a periodontal probe (UCN-15 probe).

2.4 Salivary Samples Collection:

In this investigation, the whole unstimulated saliva is obtained since it is the primary intraoral condition related to the status and composition of saliva. During the time period of 9 am to 12 pm, samples are collected in a calm area to avoid the influence of the circadian rhythm. In order to remove debris and contamination, participants are instructed to properly rinse their mouths with water before collection. Before doing a clinical evaluation, a passive drooling approach (Navazesh, 1993) is used to collect unstimulated saliva in sterile test tubes. Salivary supernatants were centrifuged at 4000 rpm for 10 min to eliminate cellular debris. Two ml of clear salivary supernatants are aspirated into a plastic Eppendorf tube using a micropipette. Labelled Eppendorf tubes are placed in a -20°C freezer until analysis.

2.5 Enzyme-linked immunosorbent assays (ELISA):

The samples are defrosted and given some time to come to room temperature. Salivary MDA levels are determined using commercial ELISA kits from (Wuhan Fine Biotech Co., Ltd). The method is carried out in accordance with the kit's manufacturer's instructions. A Microtiter plate reader (HumanReader HS; HUMAN Society for Biochemica and Diagnostica mbH) is used to measure optical density (OD). The linear regression algorithm designed specifically for the chosen biomarker is used to transform all OD data into concentrations on spreadsheets.

2.6 Statistical Analysis:

The Statistical Package for Social Science (SPSS version 21) is used for data description, analysis, and presentation. The Shapiro-Wilk test is used to verify the data distribution prior to inferential analysis. An ANOVA test is used for multiple group comparisons, along with a Dunnett T3 post-hoc test. Biomarker concentration in saliva is correlated with clinical periodontal parameters using Pearson's correlation test. Graph Pad Prism is used for figures. To evaluate the sensitivity and specificity of the biomarker, ROC curves and AUCs were used. The statistical significance was established at a significance level of $p < 0.05$.

3. Results:

3.1 Clinical findings:

In this study, (125) individuals of (20 and 65 years old) are recruited. They are split into five groups, including those with periodontal health (Control, n = 25), nonsmokers and smokers with gingivitis (n = 25 each), and nonsmokers and smokers with periodontitis (n = 25 each). The values of periodontal clinical parameters including PI, BOP, PPD, and CAL (of all groups) are summarized in (Figure 1).

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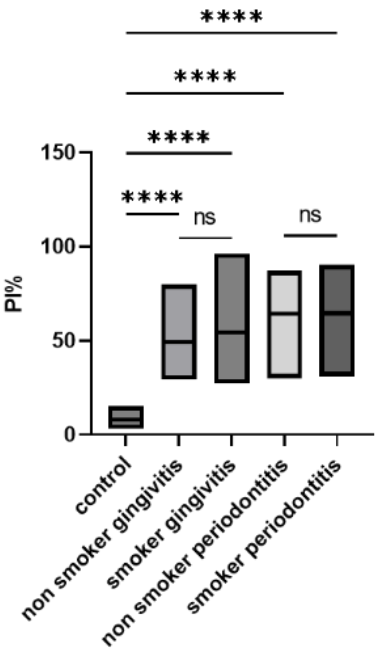
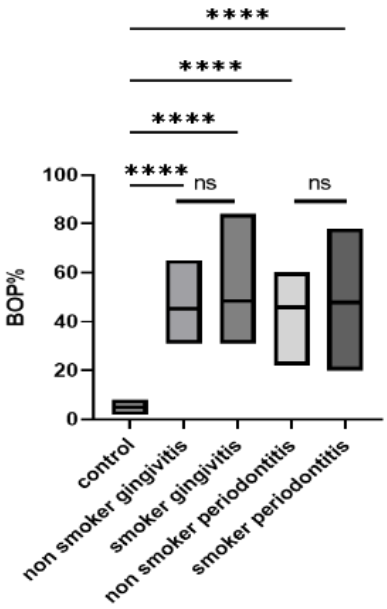
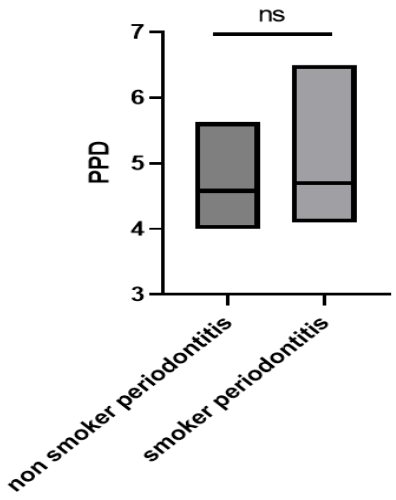
Plaque index



B) Bleeding on probing



Probing pocket depth



D) Clinical attachment loss

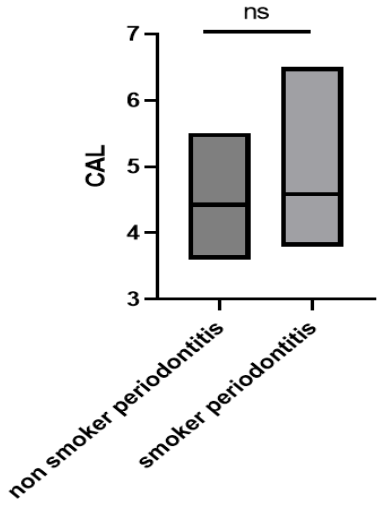


Figure 1 Comparison of, (A) PI, (B) BOP, (C) PPD, (D) CAL among study groups. *Significant difference at $p < 0.05$, ns: nonsignificant difference.

PI and BOP are considerably higher in periodontal disease groups (cases groups) compared to those in a healthy group (control) ($P < 0.05$).

However, significant variations in PI and BOP mean values between nonsmoker and smoker gingivitis groups and between nonsmoker and smoker periodontitis groups are not detected.

No significant difference in PPD and CAL is recognized between nonsmokers and smokers with periodontitis groups ($P > 0.05$).

3.2 Laboratory Findings:

Significantly elevated salivary MDA levels ($P < 0.05$) are seen in the groups of nonsmoker gingivitis, smoker gingivitis, and smoker periodontitis, as compared to the control group. Nevertheless, no statistically significant difference is observed between nonsmokers periodontitis and the control group. Additionally, the smoker groups exhibited higher levels of MDA compared to the non-smoker groups (Figure 2).

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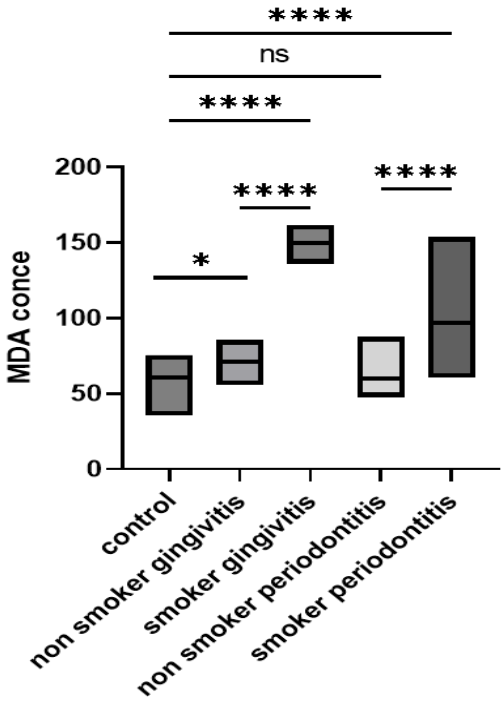
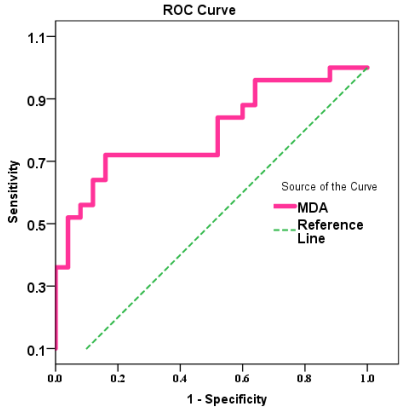


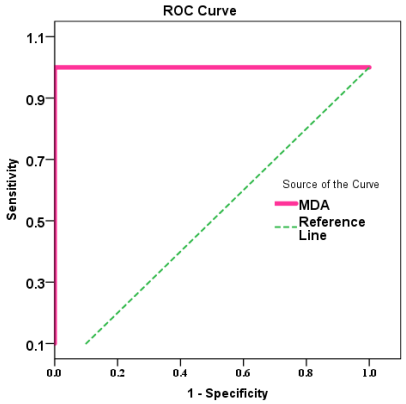
Figure 2: Comparison of salivary MDA (ng/ml) levels among Study groups, ns: non-significant. *Significant difference at $p < 0.05$. MDA, malondialdehyde.

The diagnostic sensitivity and specificity of the chosen salivary biomarker are assessed using receiver operating characteristic (ROC) curve analysis and the area under the curve (AUC) (Figure 3). MDA showed not useful to excellent AUC (AUC range 0.426 to 1) to distinguish control from smokers and nonsmokers' gingivitis and periodontitis groups(Figure3).

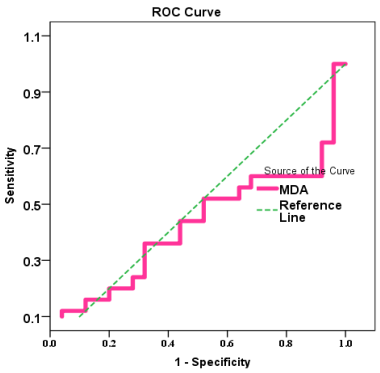
Control vs nonsmoker gingivitis (AUC:0.795)



B) Control vs smoker gingivitis (AUC: 1)



control vs nonsmoker periodontitis (AUC: 0.426)



D) control vs smoker periodontitis (AUC:0.904)

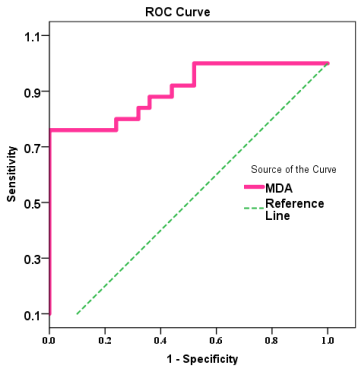


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Figure 3 Receiver operating characteristic (ROC) of salivary biomarkers: (A) control vs nonsmoker gingivitis, (B) control vs smoker gingivitis, (C) control vs nonsmoker periodontitis, (D) control vs smoker periodontitis. Salivary MDA and its ratio demonstrated high sensitivity and specificity in distinguishing control from nonsmoker gingivitis (AUC: 0.795 respectively). Likewise, the ratio of the above-mentioned biomarker demonstrated excellent sensitivity and specificity to distinguish control from smoker gingivitis (AUC: 1 respectively). In contrast, salivary MDA lack the accuracy to discriminate control from nonsmoker periodontitis (AUC: 0.426 respectively). Furthermore, salivary biomarkers demonstrated excellent accuracy in distinguishing between control and smoker periodontitis. (AUC: 0.904 respectively). AUC, area under the curve; MDA, malondialdehyde.

Table 1 provides the selected biomarker's proposed cut-off concentrations, sensitivity, specificity, and ability to distinguish between periodontal health and periodontal illness.

Table 1: The cut-off values, sensitivity, specificity, and area under the curve (AUC) for each group.

		MDA
Control vs nonsmoker gingivitis	AUC	0.795
	Sensitivity	84
	Specificity	60
	Cut-off value	88.13
	p Value*	0.000
Control vs smoker gingivitis	AUC	1
	Sensitivity	100
	Specificity	100
	Cut-off value	34.44
	p Value*	0.000
Control vs nonsmoker periodontitis	AUC	0.426
	Sensitivity	40
	Specificity	44
	Cut-off value	61.51
	p Value*	0.367
Control vs smoker periodontitis	AUC	0.904
	Sensitivity	100
	Specificity	100
	Cut-off value	34.44
	p Value*	0.000

Abbreviations: MDA , malondialdehyde. *Significant difference at $p < 0.05$

4. Discussion :

The salivary malondialdehyde (MDA) assay demonstrated a notable level of sensitivity and specificity in distinguishing between those with periodontal health and those who are smokers with periodontal disease. The aim of this case-control study is to assess the potential utility of salivary malondialdehyde (MDA) as a diagnostic biomarker.

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Saliva is chosen as the oral fluid of choice because it is simple to collect without discomfort for the patient, can be collected in significant quantities, and contains a variety of biomarkers that are accurate indicators of many different local and systemic diseases (Bhattarai et al., 2018).

The ELISA procedure is chosen in this study to detect the biomarkers concentrations because it is easy to use, it provides excellent sensitivity and high specificity due to the specific reaction between an antibody and antigen, is highly reproducible, and can quantitatively detect proinflammatory and anti-inflammatory cytokines (Jaedicke et al., 2016). The latter characteristic is crucial for figuring out the cut-off concentrations of the chosen biomarkers, which makes it easier to turn them into a clinical tool for chairside use.

Lipid peroxidation (LPO) is widely recognized as a significant outcome of free radical activity. MDA, an aldehyde compound, is among the several aldehydes generated as a result of lipid peroxidation, representing its final consequences, can be used to evaluate tissue loss by oxidative stress (Naresh et al., 2019). According to the findings of a meta-analysis, there are significant differences in the levels of the LPO biomarker MDA in patients with periodontitis and in healthy subjects. The findings confirmed that periodontitis and LPO-related biomarker levels are closely associated, demonstrating the crucial role that oxidative stress plays in periodontal disease (Mohideen et al., 2023).

In comparison to the control group, the findings of this study demonstrated that MDA levels increased in the diseased groups and that smoker groups also showed higher MDA levels when compared to nonsmokers. This is consistent with the findings of Garg *et al* (Garg et al., 2006) who noted greater MDA levels in patients with periodontitis, particularly smokers.

Moreover, these findings are similar to those of a study by Guentsch *et al*. (Guentsch et al., 2008), which reveal that smoking and periodontitis both induce a significant increase in LPO in saliva. Additionally, Tsai *et al*. (Tsai et al., 2005) observed that the levels of lipid peroxidation(LPO) in gingival crevicular fluid (GCF) and saliva are found to be significantly elevated in diseased areas compared to healthy areas and came to the conclusion that in periodontitis, there is an imbalance between oxidative stress and antioxidants, which increases the amount of tissue damage caused by ROS.

The results of the current study are also in accordance with a previous study conducted by Naresh *et al*. (2019), who demonstrated that smokers with periodontitis had MDA levels that were higher than nonsmokers with periodontitis and with healthy participants, respectively (Naresh et al., 2019).

The coexistence of periodontitis and smoking is found to lead to a notable elevation in MDA levels when compared to those observed in control subjects with healthy periodontium. The measurement of malondialdehyde (MDA) as a biomarker is a widely investigated indicator of (LPO), indicating that the extent of oxidative stress is elevated in pathological states compared to healthy settings (Naresh et al., 2019, Miran and Akram, 2023).

One of the constraints encountered in this study related to the inability to assess the female population due to their relatively low incidence of smoking behaviours. Nevertheless, it is necessary to do additional research that includes more extensive sample size and longitudinal studies that specifically involve female smokers in order to accurately estimate this marker in relation to periodontitis. While the prospective application of salivary biomarkers as diagnostic tools shows promise, additional clinical research is necessary to substantiate the recent findings.

5. Conclusion:

With the limitations of this case-control study, it may be suggested that the oxidative stress biomarker MDA is higher in periodontal diseases associated with risk factors such as smoking and this biomarker has the potential to be employed as a diagnostic biomarker for periodontal disease.

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