

# Levels of salivary malondialdehyde in traumatic ulcer patients



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## Abstract

**Objective:** Traumatic ulcers are oral mucosal lesions that are often encountered with the healing process of wounds involving inflammatory mediators and phagocytosis process. Phagocytosis can produce free radicals that the potential to cause cells damage and lipid peroxidation. Lipid peroxidation produce malondialdehyde (MDA) as end products. Saliva is a biological specimen can detect MDA level in oxidative stress condition. This study aims to analyse differences in levels of salivary MDA in traumatic ulcer participants compared to healthy individual subjects.

**Material and Method:** The research methods was cross sectional study. MDA levels measured in saliva of 50 subjects, aged 20-25 years. Traumatic ulcer lesion subjects and healthy individual without lesions

would be taken unstimulated saliva samples. Salivary MDA levels determined with TBARS method. Data were analyzed statistically with Mann Whitney's test ( $p < 0.05$ ).

**Results:** Showed that there were differences in salivary MDA levels in traumatic ulcer participants compared to healthy individual subjects ( $p = 0.0001$ ).

**Conclusion:** Free radicals have been implicated in the pathogenesis of oral mucosal lesions, including traumatic ulcer. Increase in MDA levels in the saliva of participants may provide a biomarker for oxidative stress in oral mucosa disease. Oil from bangle rhizome on streptococcus mutans bacterial growth observed from their inhibition zones.

**Keywords:** Malondialdehyde, Saliva, Traumatic ulcer

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## Introduction

The ulcer is a type of lesion in the oral mucosa with a prevalence of 15-30%. The most common ulcer is a traumatic ulcer caused by trauma marked by the loss of the epithelium layer due to the excavation of deeper surface tissue than epithelial tissue.<sup>1,2</sup> There have been many studies that prove the involvement of free radicals in the process of tissue damage, and the involvement of antioxidants in reducing free radicals to prevent oxidative stress.<sup>3,4</sup>

During the metabolic process, the body will produce free radicals as much as 2.5% of the total oxygen needed or 3.4 kg / 24 hours of oxygen. Mitochondria play an important role in cells as a place of cellular respiration that causes redox reactions. Non-physiological oxygen levels will be a stressor that will stimulate Reactive oxygen species (ROS), causing haemostasis or stimulation of growth, defence, and signalling of cells. If the ROS produced exceeds the body's antioxidant capacity, it will cause oxidative stress, apoptosis, and necrosis. Lesions that occur in the oral mucosa in the healing process involve inflammatory mediators such as neutrophils, macrophages, and eosinophils.<sup>4,5</sup>

The phagocytosis process that occurs is capable of producing superoxide radicals which can produce free radicals. One type of free radical

compounds namely Hydroxyl Radicals ( $\text{OH}^*$ ) can cause damage to cell structures due to its highly reactive nature. The oxidative stress that occurs can cause the expansion of cell damage when a lesion occurs.<sup>4,5</sup>

Free radicals are molecules or compounds that are reactive and stand-alone and contain one or more unpaired electrons in the outermost orbit. Free radicals will attack nearby stable molecules and pick up electrons. Free radicals that occur due to ROS can cause damage from proteins, lipids, carbohydrates, and nucleotides in tissues and can undergo chain reactions, thus causing widespread cell damage. Free radicals and antioxidants that are not balanced can cause oxidative stress by inducing lipid peroxidation that occurs due to changes in polyunsaturated fatty acids (PFA) by peroxy reactions that can be measured as levels of Malondialdehyde (MDA). MDA level examination generally uses the thiobarbituric acid reactive substance (TBARS) test.<sup>4,6-8</sup> Saliva is a biological specimen that can be used to make a diagnosis that originates from the exocrine gland, the salivary gland. Saliva contains immunoglobulin a (secretory IgA), minerals, electrolytes, buffers, enzymes, and metabolic waste substances. Saliva is called the 'mirror of the health of the organism' because it can reflect the body's current physiological conditions, including

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measuring the oxidative stress that occurs in the body due to free radicals.<sup>9,10</sup>

This study was carried out to compare the MDA levels of saliva in normal patients and patients with oral mucosal lesions of traumatic ulcers.

## Material and Methods

This study was carried out between January-March 2020 in the Dental and Oral Education Hospital Universitas Jenderal Achmad Yani Cimahi Indonesia. Fifty subjects of both genders were recruited. The subjects divided into 30 normal participants without any oral mucosal lesions and 20 participants who were diagnosed with traumatic ulcers. All subjects had a systemically healthy. Inclusion required subjects to have a negative smoking and recreational drug-use history and no special dietary requirements. Traumatic ulcers subjects had oral mucosal lesion due to physical trauma such as brushing teeth trauma. Exclusion criteria included use of non-steroidal anti-inflammatory or antimicrobial drugs, or use of mouthwash, or antioxidant supplements, or vitamin supplements within a 3-month period before the study commenced. Informed consent to participate was initially obtained, followed by completion of a medical questionnaire. Only those subjects who fulfilled all inclusion. This study was in agreement with the ethical principles of the World Medical Association Declaration of Helsinki (2002) and CIOMS (2016) and approval was granted by Medical Research Ethics Committee of the Faculty of Medicine, Universitas Jenderal Achmad Yani at Cimahi Indonesia, which has been approved with approval number 009/UMI.01/2020.<sup>11,12</sup>

## Clinical Procedures

Clinical measurement was performed immediately before unstimulated whole saliva collection. To determine the traumatic ulcers of the study subjects, the following assessments were recorded from anamnesis to intra oral examination.<sup>13</sup> Patients with traumatic ulcers who have agreed as research respondents must meet the following inclusion criteria: patients are competent, patients with a healthy systemic condition, ulcers due to mechanical trauma such as biting and brushing teeth, painful ulcers, ulcers for more than 3 days. Traumatic ulcer area measurement is done by measuring the outer limit of the largest length size times the largest width of the ulcer using a periodontal probe.

## Oral Cavity Examination

This check must be carried out systematically using the same order each time. We have to check the oral

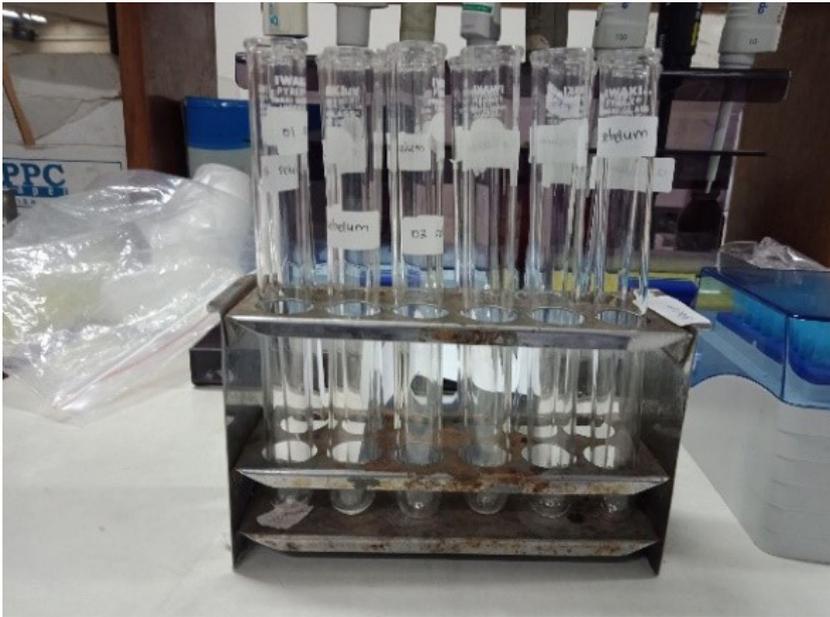
hygiene status used Oral Hygiene Index Simplified, Examination of the labial mucosa, buccal mucosa, mole palate, hard palate, the floor of the mouth by palpation. The uvula is noted to be too long or too short, or the uvula looks asymmetrical at rest. Sometimes a person is found with bifid / gaps in the uvula. The tongue is examined by looking directly or indirectly. Grasp the tip of the tongue using gauze and move the same fingers and thumb on one side to observe the lateral border then repeat for the other side. Use the mouth glass to examine the posterior lateral border of the tongue.<sup>14</sup>

## Saliva Sampling and Processing

The method of unstimulated saliva collection for each participant conducted in examination room. Saliva collection were held between 8:00 and 9:00 in the morning. All participants had been an overnight fast of at least 8 hours and were asked not to drink, except mineral or tea water for the same period. Subjects began by rinsing their mouths thoroughly several times with mineral water and then resting quietly for 3 min. Unstimulated whole saliva (4 ml) was collected. The collection trial started with the instruction to rid the mouth of saliva by swallowing. Subjects then spit the accumulated saliva into a sterilized test tube every one minute. Saliva was collected for 5 minutes. Saliva sample replaced into an Eppendorf tube and then store it at <10°C.

## Biochemical Assay

Salivary MDA levels were assayed in Biochemistry and Biomolecular Laboratory Faculty of Medicine, Universitas Jenderal Achmad Yani Cimahi Indonesia. The chemicals and materials used were the acetic acid solution (20 ml acetic acid 96% in 76 ml H<sub>2</sub>O, determine pH 3.6), 2-thiobarbituric acid solution (dissolve 0.8 2-thiobarbituric acids with 7 ml NaOH 1N add H<sub>2</sub>O to 100 ml, determine pH 3,6), Butylated hydroxytoluene (BHT) solution (44 mg butylated hydroxytoluene in 5 ml absolute ethanol), EDTA solution (1,488 g EDTA in 50 ml H<sub>2</sub>O always made new), and standard solution. Procedure for inputting 400 µl of saliva into a test tube, added 300 µl aquadest, 200 µl SDS solution, 50 µl BHT solution, 50 µl EDTA solution, 1.5 ml acetic acid solution, and 1.5 ml TBA solution **Figure 1**. Incubate in a water heater at 100 °C for 30 minutes and cover each tube with marbles. Then each tube is immersed in a tub of ice water, then centrifuge at a speed of 3000 rpm for 10 minutes **Figure 2**. Take a supernatant from each tube and read the absorbency with a spectrophotometer at λ 532 nm **Figure 3**.<sup>15</sup>

**Figure 1. Sample in the reagent****Figure 2. Centrifuge the sample process** **Figure 3. Absorbance rating**

### Data Analysis

The analysis conducted must be in accordance with the type of research problem and the data used is to determine differences in levels of malondialdehyde (MDA) in the saliva of traumatic ulcer patients and patients with normal saliva. Then for numerical data, before statistical tests the numerical data are assessed by normality tests using the Shapiro Wilk test. If the data is less than 50, the alternative is Kolmogorov Smirnov if the data is more than 50, where this test is used to test whether the data is normally distributed or abnormal distribution. Furthermore, statistical analysis matches the research objectives and hypotheses. Significance test to compare the characteristics of the two

research groups used unpaired t test if the data were normally distributed and Mann Whitney test as an alternative if the data were not normally distributed. While statistical analysis for categorical data is tested with the chi-square test if the Chi-Square requirements are met if not met then the Exact Fisher test is used for the 2x2 table and Kolmogorov Smirnov for tables other than 2x2. The requirement for Chi Square is that there is no expected value of less than 5, as much as 20% of the table.

The significance criteria used are the value of p, if  $p \leq 0.05$  is significant or statistically significant, and  $p > 0.05$  is not significant or not statistically significant. The data obtained is recorded in a special form and then processed through the SPSS program version 24.0 for Windows.

### Results

#### Comparison of salivary MDA levels of traumatic ulcer patients and saliva of healthy patients

As shown in **Table 1** describes the overall characteristics of the subjects of the patient based on malondialdehyde levels. The average level of malondialdehyde as a whole is  $12.26 \pm 18,227 \mu\text{m}$ .

**Table 1. Contents of MDA characteristics**

Variable	N=50
Levels of Malondialdehyde	
Mean $\pm$ Std	12.26 $\pm$ 18.227
Median	0.84
Range (min-max)	0.05-57.00

The results from **Table 2** show that the Malondialdehyde level in the healthy saliva patient group had an average of  $0.57 \pm 0.413$  while the Malondialdehyde level in the traumatic ulcer traumatic saliva patient group had an average of  $29.80 \pm 17.796$ . The difference in the level of traumatic ulcer patients with greater saliva compared with healthy saliva shows that the traumatic ulcer wound state increases MDA levels which indicate an increase in free radicals more in patients with traumatic ulcer, as shown in **Figure 4**.

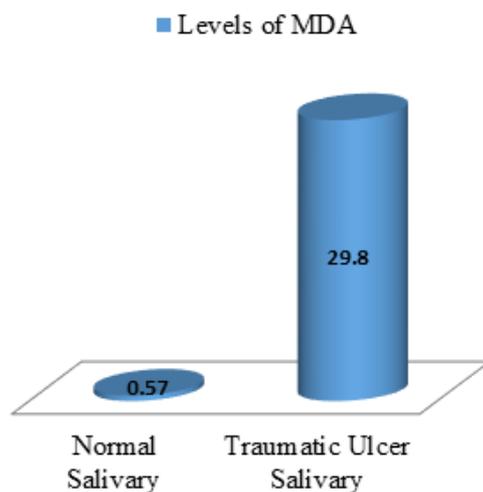
### Discussion

Free radicals are molecules or groups that standalone that contain one or more unpaired electrons in the outermost orbit, free radicals are very active by attacking the stable atoms associated

**Table 2.** Contents of comparison between malondialdehyde in the group of normal salivary patients and traumatic ulcer saliva patients.

Variable	Group		P Value
	Saliva in normal patient N=30	Saliva in patient with traumatic ulcers N=20	
MDA Levels			0.0001**
Mean±Std	0.57±0.413	29.80±17.796	
Median	0.45	24.00	
Range (min-max)	0.05-1.82	11.00-57.00	

Note: For numerical data the p value is tested by an unpaired T test if the data is normally distributed with an alternative to the Mann Whitney test if the data are not normally distributed. Significance value based on the value of  $p < 0.05$ . Sign \* indicates the value of  $p < 0.05$  means significant or statistically significant.

**Figure 4.** Concentration of salivary malondialdehyde levels in participants with traumatic ulcers in comparison with healthy control subject.

of free radicals in the body and antioxidant levels are not balanced, oxidative stress can be trusted through the levels of Malondialdehyde (MDA). Antioxidants are free defences or radical absorbers, which can be rotated through a cycle that can support the polymer to free radicals which further prevents molecules from wandering around our cells to cause damage to biomolecules.<sup>16</sup>

The process of free radicals formed is divided into two primary stages, the process of radical formation and destructive same. Formation of free radicals can occur in haemolytic cleavage or from electron transfer reactions. In chemistry, free radicals take part in the addition of radicals and radical substitution as reactive intermediates. Chain reactions that involve free radicals can usually be divided into three different processes: initiation, propagation, and stopping. Initiation is the process of forming lipid radicals by free radicals forming and attacking lipids. The next stage that occurs is the formation of peroxy lipid radicals because the

process of radical lipid reaction with oxygen molecules and takes other hydrogen molecules to form lipid peroxides. Propagation reactions involve free radicals where the amount of free radicals remains the same. The termination stage occurs when a radical compound reacts with other radical compounds or by radical capture so that the potential for propagation is low.<sup>4,17</sup>

The physiological oxygen metabolism of the body will produce the main pathway for the formation of ROS in the form of superoxide radicals, hydroxyl radicals, and non-radical H<sub>2</sub>O<sub>2</sub>. Highly reactive hydroxyl radicals can react with molecules in the body such as proteins, lipids, DNA, etc. Free radicals that cause chain reactions can induce some damage to the body's smallest molecule, DNA that can cause cancer and other diseases, so the results of several studies and this research show evidence of free radicals' role in the damage that occurs in the body.<sup>18,19</sup>

Free radicals in the pathogenesis of the disease. When a depressed organism becomes unable to neutralize excessive levels of endogenous free radicals, these substances are highly reactive irreversible cell structure damage and induce mutations involved in the pathogenesis of several diseases. Oxidative stress plays an important role in accelerating the aging process and in the development of chronic and degenerative diseases.<sup>20,21</sup>

Existing ROS due to increased free radicals with decreased antioxidants can cause oxidative stress that occurs due to oxygen metabolism, oxygen reperfusion injury due to haemoglobin, hypoxia, and myoglobin oxidation. ROS plays a role in the body's defence system, especially during the inflammatory process when phagocytosis occurs. Leukocyte cells during the phagocytosis process use 70-90% oxygen. Increased MDA levels in saliva of patients with traumatic ulcers caused by the wound

healing process, especially in the inflammatory and proliferation phase involving neutrophils and macrophages in the phagocytosis process, so that ROS involvement will be more numerous than MDA levels in normal patient saliva. The wider a traumatic ulcer, the higher a Salivary MDA value<sup>19,22-25</sup>

## Conclusion

Free radicals have been implicated in the pathogenesis of oral mucosal lesions, including traumatic ulcer. Increase in MDA levels in the saliva of participants may provide a biomarker for oxidative stress in oral mucosa disease. Further studies on a large series should be performed to clarify the exact role of MDA levels in other oral mucosa disease and to evaluate the effect of medical therapy.

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## Conflict of Interest

The authors report no conflict of interest.

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