Measurement of Malondialdehyde in Patients with Recurrent Aphthous Stomatitis

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Abstract

Recurrent Aphthous Stomatitis (RAS) is one of the most common mucosal diseases of the oral cavity. Several RAS trigger factors are suspected of creating an imbalance between oxidant and antioxidant (AO) reactions, thereby accelerating the formation of free radicals. Malondialdehyde (MDA) is widely employed as an indicator of increased lipid peroxidation, a process in which Reactive Oxygen Species (ROS) are also involved. The imbalance between ROS and AO will cause oxidative stress (OS). Saliva offers more advantages as a biological liquid biomarker in promoting early diagnosis or prognosis.

To determine the level of MDA resulting from OS reaction due to the presence of free radicals in the saliva of RAS patients.

This study constitutes pre-experimental, clinical and laboratory-based research incorporating pre-post treatment variation. The research population consists of RAS out-patients attending the Department of Oral Disease, Faculty of Dental Medicine, Universitas Airlangga. Sampling of RAS patients' saliva was carried out by means of random sampling, while the ELISA method was utilized to measure MDA levels.

An increase in the MDA levels of RAS patients was detected compared to those of their recovering counterparts.

OS reactions occurred during the ulceration stage experienced by RAS patients. This was demonstrated by an increase in MDA in the saliva of RAS patients, coinciding with ulcerated lesions, compared to that of recovering RAS patients.

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Introduction

Recurrent Aphthous Stomatitis (RAS) is one of the most common oral mucosal diseases. The etiopathogenesis of RAS has yet to be explained, but a number of researchers and specialists in the field of oral diseases consider the predisposing factors to include: immunological,immunodeficiency, hematologic deficiency, allergies, psychological stress, local trauma, tobacco consumption, genetic factors, and hormonal changes.[1] Previous study conducted by Auerkari et al. (2017) stated that one of the triggering factors assumed to contribute to RAS pathogenesis is an endocrinal or hormonal imbalance. Esterogen Receptor β expression in oral mucosa is related to the severity of minor RAS.[2] Predisposing factors of RAS may result in disruption of oxidant and antioxidant (AO) reactions resulting in accelerated free radical formation.[3]

Free radicals are molecules with unpaired electrons in their outer orbits rendering them extremely unstable and reactive.[4] Some reactive molecules and free radical derivatives of oxygen molecules are referred to as Reactive Oxygen Species (ROS), whereas reactive molecules and free radicals of nitrogen molecule derivatives are termed Reactive Nitrogen Species (RNS).[5]

ROS play a role in the survival of a species which can either threaten or contribute to the viability of a living system. If ROS production is high, but ROS and AO are out of balance, this can potentially cause cell damage and provoke a

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reaction referred to as oxidative stress (OS). In the cell metabolism process, damage caused to the cell membranes and lipoproteins by ROS is a process known as lipid peroxidase within which a radical bond reaction involving lipid molecules occurs.[6] As a result of the lipid peroxidase process, a final product, Malondialdehyde (MDA) is obtained and widely used as an indicator of increased lipid peroxidation involving both ROS and OS.[7] Several studies have linked OS improvement with low AO capacity in RAS patients. Research on OS and AO has previously been undertaken by Cimen et al. (2003) and included the measurement of serum AO vitamin and lipid peroxidation in RAS patients.[8,9]

Saliva has more value as a biological fluid and biomarker in supporting a diagnosis or prognosis. Samples can be collected by means of a non-invasive and pain-free experimental procedure which can be repeated several times a day in order to enhance accuracy.[10] Saliva contains many enzyme derivatives originating from the salivary glands, epithelial cells and ulcerated lesions of RAS patients.[9]

The purpose of this study is to determine MDA levels resulting from OS reactions caused by the presence of free radicals in the saliva of RAS patients.

Materials and methods

This research was approved by ethical clearance from the Committee of Ethical Clearance of Health Research, Faculty of Dentistry, Universitas Airlangga (No. 188/KKEPK.FKG/XI/2013). It was conducted at the Oral Medicine Clinic, Faculty of Dental Medicine, Universitas Airlangga and the Laboratory of the Institute of Tropical Diseases, Universitas Airlangga Surabaya between November 2013 and January 2014, incorporating analysis of eight samples. This research constituted a combination of pre-experimental, clinical and laboratory-based investigation with some variations of pre-post treatment. The study population consisted of RAS patients who attended the Oral Medicine Clinic at the Faculty of Dental Medicine, Universitas Airlangga. The research sample criteria applied were as follows: 1) patients drawn from both genders 2) within the 15-40 years age range 3) a history of a recurrent ulcer(s) with an optimum frequency of once a month and a minimum frequency of twice a year 4) single or multiple ulcers [up to a maximum of five] 5) ulcers in the 2nd-4th post-presentation day and 6) ulcers with a diameter of 2-9 mm. The other inclusion criteria comprised the absence of a history of systemic disease or symptoms of systemic disease in the oral cavity, the lack of resort to medications or therapies potentially causing oral mucosal changes, and freedom from acute or chronic infectious diseases.

Saliva sample collection was executed by means of a random sampling technique involving the use of a Human Malondialdehyde ELISA kit. Saliva collection was repeated twice, the first time when RAS had occurred and the second after the healing process was complete. The supernatant was extracted from the whole saliva sample and stored at -40ºC. The saliva was then processed by ELISA method. Data analysis was completed using a paired T-test with 95% significance level.

Results

Time	Average level of Malondialdehyde (ng/ml)	Standard Deviation	Kolmogorov Smirnov Significance
At the time of *RAS* (n=8)	1,9675	0,59093	0,132
Healing RAS (n=8)	1,8138	0,54100	0,200

Table 1. The mean rate of Malondialdehyde at the time of RAS and post-healing.

	Time	Mean Value	Standard Deviation	Sample t-test Significance
Malondialdehyde Level (ng/ml)	At the time of RAS (n=8)	1,9675	0,59093	0,312
	Healing RAS (n=8)	1,8138	0,54100	

Table 2. Differences between Malondialdehyde levels during RAS and post-healing.

Levels of MDA increased at the time of RAS compared to the post-healing period. Based on the results, data distribution was normal (p> 0,05) as can be seen in Table 1. Analysis of data using a paired t-test revealed there to be no significant difference in the MDA level during RAS. After the condition had been cured, this was apparent based on the significance value of the MDA level being greater than 0.05. It was 0.312 (p> 0.05), as can be seen in Table 2.

Discussion

RAS constitutes one of the chronic inflammatory diseases of the oral mucosa. RAS

presents clinical feature, such as recurrent ulcers, oval or round, sick, single or multiple, with an erythematous edge.[11] Several recent studies suggest that, in the presence of precipitating factors of RAS, both local and systemic factors contribute to the RAS pathogenesis, either directly or indirectly related to the balance of oxidants (ROS) -OO in the body.[3] The alleged role of ROS and AO in protecting against RAS pathogenesis has been investigated. An increase in ROS exceeding physiological values may give rise to the OS.[12] The measurement of MDA levels in this study used saliva as the sample. Saliva is a major determinant of the oral environment that can be used as a diagnostic tool in easily accessible systemic conditions. Currently, several studies are intensely researching saliva as one of the diagnostic tools.[10]

In this study, the measurement of OS levels involved MDA as a marker. MDA as a preview of the OS is a form of degradation of lipid peroxidase caused by ROS.[7] Measurements of MDA levels were performed using RAS patients' saliva during and after the ulcer had been cured. OS and MDA levels increased in RAS patients. A previous study conducted by Arikan et al, revealed that MDA levels increased in RAS patients as the OS precursor, compared to healthy patients without RAS. Increased OS levels may occur due to an imbalance in the systemic conditions of ROS. The body has the ability to heal and detoxify tissue damage caused by reactive molecules or free radicals. The cytotoxic effects caused by free radicals can trigger the deterioration of normal cell function and cell structure integrity through peroxidative destruction in the double chains of fatty acids, proteins, and DNA. OS inhibit the immune system's ability to protect cells or tissues from damage. OS also causes certain inflammatory diseases in the soft tissues of the oral cavity.[4,8,13,14]

Conclusions

MDA levels increased in RAS patients to a greater extent during the ulceration phase than after the healing phase. Therefore, OS is recommended as an RAS pathogenesis.

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Declaration of Interest

There is no conflict of interest in this study.

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