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ASSESSMENT OF THE SALIVARY LEVEL OF SUPEROXIDE DISMUTASE AND MELATONIN IN LOCALIZED PERIODONTITIS VERSUS GENERALIZED PERIODONTITIS

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Abstract

Background : Periodontitis is caused by a microbial invasion and an inappropriate immunological reaction of the host One of the mechanisms in the etiopathogenesis of periodontitis is alterations in the local and/or general markers of oxidative stress. In order to defend themselves from the action of oxygen-free radicals, Organisms that use oxygen in their cellular metabolism are supplied with defense systems. The term "the system's antioxidative barrier" is frequently used to describe them. superoxide dismutase (SOD) is one of the enzymatic antioxidants that preserves the cell against reactive oxygen species by removing superoxide radicals and hydrogen peroxide, Melatonin is natural hormone in the body with antioxidant effects.

Aims: T he aim of this study is to evaluate the salivary levels of superoxide dismutase (SOD) and Melatonin in patients with localized periodontitis versus generalized periodontitis.

Patients and Methods: Whole saliva samples were collected from 90 patients that divide into three groups: 35 generlized periodontitis , 35 localized periodontitis ,and 20 healthy subjects, Salivary samples were collected from patients prior to clinical examination, Then levels of (SOD) and melatonin in saliva were determined spectrophotometrically using enzyme linked immunosorbent assay (ELISA).

Results : salivary levels of both SOD and MLT were highest in control group and decreased in Localized periodontitis and generalized periodontitis with lowest level in generalized periodontitis with significant differences between groups.

Conclusion: Both localized and generlized periodontitis are associated with a decrease level of antioxidants

Key Words: Antioxidants, Melatonin, Oxidative Stress, Periodontitis, , Saliva, Superoxide Dismutase

Introduction

The irreversible loss of tissues that hold the teeth is a hallmark of the inflammatory disease known as periodontitis. Microbial biofilm deposition on teeth is what causes and maintains it, and aberrant host immune responses to harmful bacteria in periodontal pockets make it worse [1], It has been shown that the local and/or systemic signs of oxidative stress may be associated to periodontitis [2]. a state known as oxidative stress develops, When reactive oxygen species levels are out of harmony with the host's antioxidant defenses, oxidative stress occurs, causing DNA, lipid,

and protein damage [3]. Polymorphonuclear leukocytes (PMN) play a key role in the host defense mechanism against spreading periodontal pathogenic bacteria during the pathogenesis of periodontitis. Periodontal tissues are destroyed as a result of reactive oxygen species (ROS) produced in great amounts by activated PMN[4]. In health, the antioxidant defense system neutralizes free radical and nonradical species[5].

One of the enzymatic antioxidants that defends the cell from the harmful effects of ROS is superoxide dismutase (SOD). Reactive O2 is transformed by SOD into H2O2. The next process involves salivary enzymes converting H2O2 into H2O and O2 [6]. The catalytic conversion of superoxide is carried out by SOD.SOD1, SOD2, and SOD3 are the three different types of superoxide dismutase that are found in humans. SOD1 is a dimer (consists of two units), and it is found in the cytoplasm. SOD2 is a tetramer (four subunits), and it is found in the mitochondria. SOD3 is also a tetramer, but it is extracellular[7].

SOD Levels in periodontal ligament was found to be much lower than that in red blood cells and to have decreased with aging. Early in the 1990s, periodontitis patients showed spontaneous superoxide production in gingival crevicular fluid (GCF) and increased superoxide production by PMNs.[8].Melatonin is an indolamine that is primarily made by the pineal gland. It possesses antioxidant and immunomodulatory properties. It is a powerful antioxidant that defends against cellular destruction and inflammation brought on by reactive oxygen species. Additionally, it has a potent angiogenic effect that enhances the benefits of melatonin[9]. The role of melatonin both in physiological and pathological processes related to periodontitis is basically due to its antioxidant and anti-inflammatory effects, as well as it acting as a mediator in bone formation and resorption [10]. Salivary melatonin levels are inversely correlated with the severity of periodontal disease. As a result, older adults' lower melatonin synthesis, which is reflected in low levels of salivary melatonin, and their decreased salivary output with aging increase their risk of developing periodontal and oral disease, respectively[11]. Clinical parameters are commonly used to identify periodontal disease (alveolar bone loss and clinical attachment levels). These criteria, however, are unable to reveal the disease's current state. In other words, these parameters cannot detect disease before a significant amount of damage has already occurred, Numerous chemicals found in saliva that derive from either the host or bacteria have been considered as potential indicators for periodontal disease [12]. Therefore, saliva is a clinically useful (biofluid) that is used to detect disease, assess its progression, monitor and treat patients with oral diseases[12&13] .

Patients and methods

Ninety patients (both male and female) requesting periodontal treatment at the Department of Periodontics in the teaching hospital of the College of Dentistry, University of Baghdad, served as the study's subjects. The subjects' ages ranged from 28 to 50. The research ethics committee of the College of Dentistry/ University of Baghdad approved the protocol and A signed informed consent was provided to all participants. According to the inclusion criteria, subjects should be in good overall health, have at least 20 teeth, and not have used antibiotics or anti-inflammatory medications during the previous three months. The exclusion criteria included patients who have recently had or are undergoing intensive therapy. either a smoker or a drinker, a patient who refuses to participate , patients taking vitamin C and other antioxidant medicines. individuals with long-term systemic illnesses,

immunocompromised patients, pregnant , women taking birth control tablets, and nursing mothers. Patients who suffer from ulcers, white lesions, and red lesions on the soft and hard palate, respectively. Patients sporting braces, dentures that are removable, implants, crowns, and bridges. those who have caught the active Corona virus. Three groups were created from the study subjects.

-Group A Control clinically healthy intact periodontium: composed of 20 subjects without probing attachment loss, no Probing pocket depths ≤3 mm, no Bleeding on probing, no radiological bone loss (Tonetti et al., 2018).

-Group B localized periodontitis stage III: composed of 35 subjects in which Interdental bone loss involving middle third of root (<30 % OF the tooth involved) (Tonetti et al., 2018).

-Group C generalized periodontitis stage III: composed of 35 subjects in which Interdental bone loss involving middle third of root (>30% of teeth involved) (Tonetti et al., 2018). Patients in these groups were asked to avoid eating or drinking for 1-2 hours and to rinse their mouths with tap water before salivary samples were taken. After collecting a saliva sample, the clinical periodontal parameters (plaque index, bleeding on probing, probing pocket depth, and clinical attachment loss) were examined on six surfaces using the Michigan O periodontal probe.Whole saliva was expectorated into sterile tubes that were labeled with the subject's number and placed in a small cooling box after collection to prevent bacterial growth. To separate the dead cells from the salivary supernatants, samples were centrifuged (Thermo scientific, Pico 17 centrifuge, Massachusetts, USA) at 4000 rpm for 3 minutes. After centrifuging to remove the cellular debris, the sample was aspirated once more and kept in a clean Eppendorf tube. It was then frozen at -200c until it could be examined using an enzyme-linked immunosorbent assay (ELISA). By using a kit manufactured by SHANGHAI YEHUA Biological Technology Co., Ltd using ELISA technique The biochemical analysis of salivary SOD and MLT were done.

Results

The results of this investigation showed a significant difference between the groups, with salivary levels of SOD and MLT being highest in the control group compared to LP and lowest in GP. as showed in(Table I) that demonstrated the statistical analysis of salivary superoxide dismutase and melatonin in periodontally healthy controls and subjects with periodontitis (GP and LP), where the level of SOD in LP (59.713U/L) higher than GP (56.750 U/L). And the level of MLT in LP (69.13) higher than GP (53.80). Following multiple pairwise comaparison used Hochberg GT2,

Table I: Descriptive and statistical test of SOD (U/L) and MLT (ng/L) among groups.

Groups	SOD Mean ±SD	MLT Mean ±SD
Control	94.406±6.968	95.99± 7.08
LP	59.713±9.073	69.13±17.25
GP	56.750±10.830	53.80±11.12
P value	0.000 sig	0.000 sig

Table II: Multiple pair wise comparisons of SOD among groups using Hochberg GT2.

Dependent Variable		(I) Groups	(J) Groups	MD	p value
SOD	Hochberg GT2	Control	LP	34.693	0.00000
			GP	37.655	0.00000
		LP	GP	2.963	0.46452

Table III: Multiple pair wise comparisons of Melatonin among groups using Games-Howell.

Dependent Variable		(I) Groups	(J) Groups	MD	p value
MLT	Games-Howell	Control	LP	26.86	0.00000
			GP	42.19	0.00000
		LP	GP	15.33	0.00011

Table IV : Correlations of SOD and MIT among groups.

Groups		SOD	
		r	p
control	melatonin	0.068	0.783
LP	Melatonin	0.30	o.o7
GP	melatonin	0.48	0.003*

DISCUSSION

The study's findings demonstrated that salivary level of superoxide dismutase was highest in control group and lower in LP with lowest level in GP with significant differences, These Results agreed with [15&16], and disagree with [17] where Patients with periodontitis had higher levels of SOD in their saliva than those in the control group. Among the most common antioxidant enzymes in the body is superoxide dismutase (SOD) [18&19]. One of its functions is the conversion of superoxide anions into hydrogen peroxide (H2O2), which works as a preventative antioxidant by preventing the hydroxyl radical from forming (OH) [20]. More superoxides were produced as a result of increased ROS generation in periodontitis, which also increased periodontal inflammation. By promoting the dismutation of two O2 to H2O2, SOD serves to remove toxic ROS from the cellular environment., hence more SOD would be consumed to overcome these radicals, causing a decrease in SOD levels [21].

The current study also showed that salivary melatonin level was highiest in control group and lower in LP and lowest in GP group with significant difference. The results in

accordance with [22-26] where healthy people had salivary melatonin levels that were higher than those of patients with periodontitis [27] who found that Salivary melatonin levels were higher in those with periodontitis and gingivitis compared to healthy. In periodontitis group there was a significant difference between GP and LP, this explained by in GP there was more destruction sites than LP so more oxidative stress, Melatonin, a hormone with antioxidant effects, may effectively treat chronic illnesses by minimizing oxidative stress. [28]. Melatonin may take part in the antioxidant defense directly by eliminating free radicals [29&30]. Melatonin may support the anti-oxidative defense indirectly by inhibiting the development of ROS-producing enzymes or directly by capturing free radicals [31]Melatonin levels consequently decreased and were worn out. The level of periodontal disease affects the salivary melatonin levels. Salivary melatonin levels decreased as periodontal disease severity increased, suggesting that melatonin may function to defend the body from bacterial infections. Consequently, melatonin may be useful in the management of periodontal infections [11]. according to Pearson's correlation coefficient results of this study showed a positive weak significant association between melatonin and SOD in the periodontitis group, This positive correlation is explained by the antioxidant properties of melatonin, which act as scavengers and indirect antioxidants to alleviate the oxidative stress caused by periodontal infection [11], and might reduce periodontal tissue's inflammatory response and tissue loss, Melatonin works through a number of different mechanisms. MLT and its metabolites interact with intracellular proteins, such as calmodulin nuclear membrane receptors of the RZR/ROR family, and receptors at the cell membrane, such as MT1 and MT2, to act as direct scavengers of free radicals and as an indirect antioxidant [32]. Superoxide dismutase is a preventive antioxidant that works by removing superoxide and hydrogen peroxide ions from the environment. Superoxide anion (O2) is efficiently and selectively removed from the body by the major antioxidant enzyme SOD, which catalyzes its dismutation into H2O2 and O2. [33].

CONCLUSION

Both localized and generlized periodontitis are associated with a decrease level of antioxidants.

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All authors listed have made a substantial, direct, and intellectual contribution to the work, and approved it for publication.
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The corresponding author is prompt to supply datasets generated during and/or analyzed during the current study on wise request.

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