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RESEARCH ARTICLE

Assessment of Oxidative Stress and Antioxidant Levels in Chronic Periodontitis Patients

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ABSTRACT

The aim of the present study was to assess the oxidative stress and antioxidant levels in periodontal patients. Chronic periodontitis patients and a control group were compared for SOD and MDA levels using TBARS and pyragallol auto-oxidation method and data was analysed. It was found that oxidative stress was higher in the study group when compared to control group and antioxidant levels were higher in the control group when compared to study group. A balance between the oxidation and anti-oxidant reactions is to be maintained in the body. The cells and the body are protected by antioxidants which guards them against the free radicals. In inflammatory conditions, this balance is broken. Hence, chronic periodontitis patients have high levels of oxidative stress and low levels of anti-oxidants. MDA and SOD can be used as a measure to find the oxidative stress in periodontal patients.

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Introduction

Chronic periodontitis is an inflammatory disease set off by Gram-negative bacteria residing in the sub-gingival biofilm (Liu *et al.*, 2014). The biofilm starts of as a pellicle which is essentially a protein coating on the tooth surface, most of which comes from the saliva. The pellicle has a protective function, it acts as a barrier against acids secreted by the bacteria thus protecting the teeth however it is also responsible for the formation of plaque.

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The pellicle serves as a favourable site for bacterial colonisation which eventually forms a biofilm of plaque which can be present in the supra-gingival or sub-gingival area. Plaque hardens to forms calculus (Marsh, 2014). Besides causing the formation of yellow-brown masses on the tooth surface which is highly unsightly, it also causes halitosis or bad breath, gingivitis and if present chronically will lead to periodontitis. Periodontitis is one of the most common oral disease worldwide and shows high prevelance especially in developing countries (Shewale, 2016). Periodontitis is preceded often by long standing gingivitis.

Periodontitis is basically the inflammation of the periodontium often clinically presenting as bleeding and deep pockets on probing. 9 in every 10 people were found to have periodontitis, showing a prevalence of 85% in states like West Bengal, Uttar Pradesh and Assam.

Several research studies suggest that the bacterial flora in a healthy oral cavity is distinct from that of a diseased oral cavity. Some common characteristic bacteria known to cause periodontitis are *Porphyromonas gingivalis*, *Tannerella forsythia* and *Treponema denticola* (Aas J et al., 2005). These bacteria activate the host defense mechanisms (Dhotre et al., 2012) and induce the formation of reactive oxygen species (ROS) (Dahiya et al., 2013) or free radicals like superoxide and hydrogen peroxide. Free radicals in moderate levels help adapt the body to the ongoing oxidative stress but in chronic disease that produce increased oxidation of tissues and cell death leading to destruction of tissues surrounding the teeth, and thus causing mobile teeth or tooth loss.

Free radicals have one or more unpaired electrons making them unstable and therefore highly reactive. They are basically electron scavengers and will not settle until all the unpaired electrons are gained. ROS often destroy tissues by forcefully taking hold of electrons from healthy tissues such as periodontium (Liu et al., 2014). This destabilises the periodontal tissue which in turn will hunt for electrons from the surrounding tissue and set off a chain reaction of electron scavenging similar to a dominoes effect. To combat this the body has an antioxidant defence system. An antioxidant has extra electrons that it gives off making free radicals stable and un-reactive. Anti-oxidation thus controls and limits oxidation of tissues. Some common sources of antioxidants are vitamin A, C and E, fruits and vegetables high in beta carotene. Research suggests that there is a strong link between oxidative stress and the pathogenesis of chronic periodontitis (Aas et al., 2005). Hence, the aim of this study was to assess the oxidative stress and antioxidant levels in chronic periodontitis patients.

Materials and Method

60 subjects representing both genders were subjected for the study after informed consent. 30 Normal healthy individuals served as the control and another 30 individuals with chronic periodontitis served as the test group. Obese subjects, patients with systemic diabetes mellitus, cardio vascular disease, hypertension and endocrine disorders and immunocompromised persons

The reason behind excluding patient who are obese, have systemic diseases and immunocompromised state was, such patients tend to show high oxidative stress which will possibly cause higher reading for oxidative stress and inability to record oxidative stress specifically for periodontitis.

Sample Collection and Procedure

5 ml blood was collected from the participants after obtaining informed consent and was centrifuged at 2500 rpm for 10 minutes. The Serum was separated and analysed for melondialdehyde (MDA) and superoxide dismutase (SOD) by TBARS method and Pyrogallol auto-oxidation method respectively using ERBA CHEM 5 plus analyser.

TBARS method

Oxidation occurs during respiration and digestion to produce energy, in addition it gives of various ROS, malondialdehyde (MDA) is one such important oxidation product, a biomarker of lipid peroxidation. Thiobarbituric acid reacts with malondialdehyde, which gives a coloured compound, the intensity of which can be determined by various methods (Zeb, 2016).The TBARS method measures MDA levels quantitatively.

Pyragallol Auto-oxidation

This is based on the ability of the enzyme superoxide dismutase to inhibit auto-oxidation of pyragallol. It is based on the reaction of free radical and SOD. This method measures antioxidant levels quantitatively. MDA Levels measure the oxidative stress and SOD levels measure the anti -oxidant levels in the body.

Results

The data was collected and statistically analysed. The results of MDA and SOD levels in chronic periodontitis patients and healthy individuals (Table 1). Unpaired t-test was used to analyse this data. The p value was found to be significant. The mean MDA levels in healthy individuals were 1.07 ± 0.54 and that of chronic periodontitis patients was 3.1 ± 1.18. [Figure 1]. It is found that the mean SOD levels in healthy individuals is 188.38 ± 26.55 and that of chronic periodontitis patients is 92.41 ±22.59 [Figure 2].

Table 1. Mean MDA and SOD of control and chronic periodontitis patients

Parameters	Control		Chronic periodontitis		p value
	Mean	SD	Mean	SD	
MDA(nmol/ml)	1.07	0.54	3.1	1.18	<0.000
SOD (U/ml)	188.38	26.55	92.41	22.59	<0.000

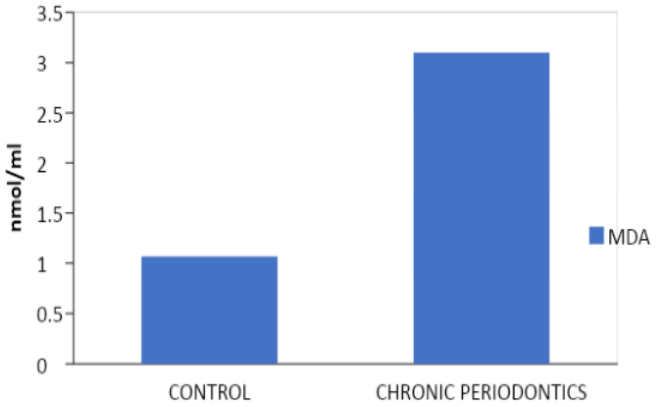

Figure 1. Mean MDA level in control and Chronic periodontitis Patients

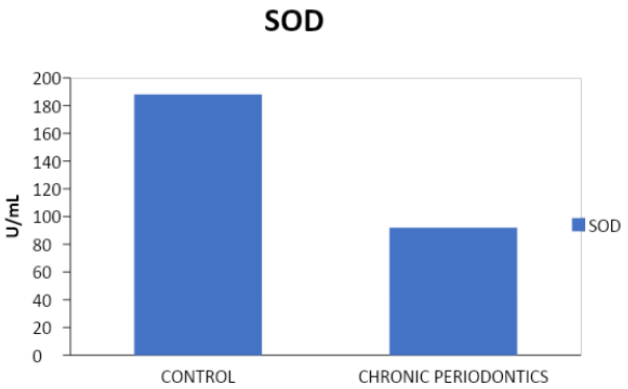

Figure 2. Mean SOD level in control and chronic periodontitis patients

Discussion

Periodontitis is the inflammation of periodontal tissue affecting a majority of people worldwide (Tonetti et al., 2015). Periodontitis comprises of three components namely cementum, alveolar bone and periodontal ligament. The health of the periodontium is key to healthy teeth. Any disease of the periodontium usually causes mobility and eventually exfoliation of teeth. Tooth loss is the hallmark of failure of a dental procedure affecting both social and emotional aspects of human health.Any dental procedure aims ultimately at saving the tooth (Plessas, 2014).Study reports show that that comprised periodontal health was the major reason for tooth extraction in people above 50 years of age (Hull et al., 1997).

In another similar study periodontal disease ranked second at 14.4%for the reason for tooth extraction. This is a huge number owing to tooth loss despite un- compromised tooth health (Jafarian, 2013).These studies therefore strongly suggest the need for thorough diagnosis and treatment for periodontal diseases. The main reason for inflammation of the periodontium is due to the presence of bacteria, in the form of plaque and calculus. The chronic and progressive infection leads to a compromise in periodontal health. Inflammation is a reparative mechanism to help the body get rid of infection (Laroux, 2014). Long-standing chronic inflammation however does more harm than good.

Periodontal diseases are known to be associated with diseases of the heart, diabetes mellitus, pregnancy, oral contraceptives and immuno - compromised state. The use of cigarettes is also known to increase the risk of getting periodontitis. A study done in the year 2014 states connection between periodontal health and cerebrovascular disease (Prejna et al., 2014).A recent study published in the year 2017 reported that ABO and Rhesus factor a possible risk factor for developing periodontitis. People with blood group O are more prone to get periodontitis based on the blood group antigens expressed (Al Askar, 2017).

Patients suffering from periodontitis have a characteristic clinical presentation often complaining of bleeding gums (Offenbacher et al., 2008), pain, swelling of gingiva, mobility, halitosis and gingival recession. On diagnosing dentists often find bleeding and deep pockets on probing. Radiographs often show bone loss surrounding the affected tooth.If more than 30% of the teeth are affected it is diagnosed as generalised periodontitis and if less than 30%of teeth are affected it is localised periodontitis.

Current treatment for periodontitis is scaling, currettage, flap surgery, full mouth disinfection (Lindhe et al., 1984) all aiming at reducing bacterial count and therefore putting an end to the inflammatory process but it is not enough to cure the disease, and therefore in many cases recurrence is seen.

Malondialdehyde (MDA) is one of the products lipid peroxidation and used to detect oxidative stress. In this study, the MDA level was 3.1±1.18 in chronic periodontitis and that in normal patients 1.07± 0.54 [Figure 1]. The antioxidant level in chronic periodontitis was 1.76±0.09 and that of normal patients was 1.15±0.18 (Ahmadi Motamayel et al., 2017). There was an increase in MDA levels in chronic periodontitis patients when compared to that of healthy individuals which confirmed the ongoing oxidation process in periodontitis owing to chronic inflammation. When cells are inflammed the antioxidant capacity of the cell is reduced or exhausted leaving cells unguarded from the free radicals. The free radicals thus attack the cells and produce oxidation products such as melanaldehyde and hydroxynonenal. Thus MDA and HDE serve as biomarkers of oxidative stress (Mittler, 2002).

Antioxidants are molecules that prevent oxidation of other molecules thus reducing the production of free radicals or reactive oxygen species. Free radicals are in search of electrons to achieve a balance of charges within and hunt for electron sources. Antioxidants help free radicals by donating electrons to them and thereby stopping the chain reaction set off by them. Antioxidants include vitamin A, Vitamin C and vitamin E, fruits and vegetables containing beta carotene like carrots, pumpkins, pomegranate and berries, lutien, Lycopene and Selenium (Chitsazi et al., 2017, Thangavelu et al., 2017) are good sources of antioxidant.

Antioxidants are known to treat various inflammatory conditions. Research suggests that chronic inflammation is what affects antioxidant levels as there is rapid exhaustion of anti-oxidants in chronic inflammation. There are various antioxidants in the body such as super oxide dismutase enzyme .It is an enzyme that catalyzes the dismutation of the superoxide (O_2^-) radical. Superoxide is produced as a by-product of oxygen metabolism. Usually there is a balance between the antioxidant and oxidation in the body, if not regulated, it can lead to cell damage. SOD is an important antioxidant defense system. In this study it was 92.41 ± 22.58 in chronic periodontitis and 188.38 ± 26.55 in normal patients [Figure 2]. Similar reports are found in other studies too. (Punj et al., 2017).

There is reduced antioxidant levels in chronic periodontitis. This can be explained in two ways. The first reason is since the body is under long standing periodontitis which is an inflammatory condition the body has exhausted or diminished the antioxidant stores therefore giving a reduced reading of SOD. The second reason could be due to the low dietary intake and body ability to produce

antioxidants therefore causing the inflammation in the first place.

Conclusion

The balance between oxidation and anti-oxidation reactions is necessary. The cells are protected by antioxidants which guards them against free radicals. When this balance is broken it leads to interaction between free radicals and normal cells, free radicals react with the cells in a process known as lipid peroxidation. Initial treatment plan focuses on the destruction of bacteria. Hence, scaling, root planning and curettage are the current lines of treatment. However, focus to balance between free radicals and antioxidant level could be the future of periodontal diseases with further research. This means giving antioxidant supplements and antibiotics together can produce a synergistic effect and help eradicate the invasive procedures employed. Further research is needed to confirm natural antioxidant therapies as definitive treatment for periodontitis.

Conflict of Interest: Nil.

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