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Evaluation of serum antioxidant and oxidative stress status in patients with chronic periodontitis

Research Project
Submitted to the department of (Education Chemistry) in partial fulfillment of the requirements for the degree of B.A or BSc. in (Chemistry)

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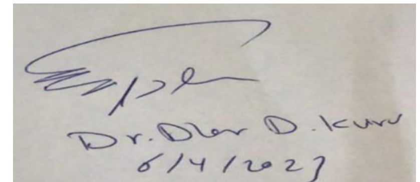
Declaration

we hereby declare that all the information in this document has been obtained and presented in accordance with the academic rules and ethical conduct. We also declare that we have sincerely followed the rules and conduct as required. we assure that we have fully cited and referenced all the material and results that are not original to this work.

Supervisor recommendation

I am the student's supervisor, ***Israa Fakher and Soma Muhammad***

I support that the student has completed all the requirements for submitting the research drawn entitled”**Evaluation of serum antioxidant and oxidative stress status in patients with chronic periodontitis”**-according to the numbered administrative order 3/1/5/1972 on 9th oct. 2022 in accordance with the instructions of Salahaddin university quality assurance and it is ready for discussion.



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We must be thankful to God, the one who improved me to add extra education to this study. Then we should be thankful to my different people in life, my beloved father who stayed ready to lose everything only to make me win. Besides, my beloved mother who stayed with me was in total pain. My great family such as brothers and sister supported me. Furthermore, all those who were with me from the first step of college down to where I am now. Feel thankful to my respectful supervisor, who is ***Dr. Parween Abdulsamad Ismail***. From the first day to the last, who was there for me with a unique study idea in the Kurdistan region. Never forget your support, kindness, and your level of teaching.

Abstract

Background: Chronic periodontitis is an infectious disease set off by Gram-negative bacteria residing in the subgingival biofilm, leading to the destruction of soft and hard tissues surrounding the teeth, and is responsible for causing tooth loss .

Aim: Local bacteria stimulate polymorphonuclear neutrophils to release reactive oxygen species in periodontitis. Increased levels of oxidative stress play a significant role in the pathogenesis of periodontitis. Therefore, this study aimed to evaluate total serum antioxidant [superoxide dismutase (SOD), Glutathione(GSH)] and malondialdehyde(MDA) in patients with chronic periodontitis.

Materials and methods: Forty healthy subjects and 45 patients with chronic periodontitis, with an age range of 30–50 years, were evaluated. After clinical examination and case selection. Blood samples were taken from the antecubital vein. Total antioxidant capacity and malondialdehyde levels were evaluated by ELIZA technique assay. Data were analyzed with t-test, using Stata.11 software program.

Results: The periodontitis group exhibited lower serum Superoxide dismutase(SOD)(92.41±22.59 U/ml) and Glutathione(4.00 ±1.33ng/mL) respectively compared to the control group(188.38±26.55U/ml);(11.47 ±3.03ng/mL) respectively. Mean serum malondialdehyde levels in the case and control groups were (3.1 ± 1.1.18 nmol/mL) and(1.07 ± 0.54 nmol/mL), respectively. The results showed significantly higher levels of serum malondialdehyde in the periodontitis group.

Conclusion: This study indicated increased levels of serum oxidative stresses in patients with chronic periodontitis. serum antioxidant (Superoxide dismutase and Glutathione were significantly lower in the serum of these patients. Hence, chronic periodontitis patients have high levels of oxidative stress and low levels of anti-oxidants. Malondialdehyde, Superoxide dismutase and Glutathione can be used as a measure to find the oxidative stress in periodontal patients.

Keywords: periodontitis, malondialdehyde, oxidative stress, antioxidants,

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LIST OF ABBREVIATIONS

reactive oxygen species……………………………………………………………(ROS)
superoxide dismutase ………………………………………………………………..(SOD)
glutathione peroxidase…………………………………………………………….. (Px)
adenine dinucleotide phosphate……………………………………………... (NADPH)
aggressive periodontitis ……………………………………………………………(AgP)
Malondialdehyde………………………………………………………………. (MDA)
Chronic periodontitis………………………………………………………… (CP)
bleeding on probing……………………………………………………………….. (BOP)
actinomycetemcomitans……………………………………………………… (AA)
subantimicrobial dose doxycycline…………………………………………… (SDD)
lipopolysaccharide……………………………………………………………... (LPS)
pattern recognition receptors …………………………………………………..(PRRs)
Toll-like receptors ……………………………………………………………...(TLRs)
microbial-associated molecular patterns……………………………………….. (MAMPs)
polymorphonuclear neutrophils ………………………………………………(PMNs)
adenosine triphosphate…………………………………………………………… (ATP)
electron transport chain ……………………………………………………...(ETC)
endoplasmatic reticulum……………………………………………………….. (ER)
8- hydroxydeoxyguanosine……………………………………………… (8-OHDG)
reactive oxygen metabolite’s ………………………………………………….(ROMs)
serum separator tubes……………………………………………………….. (SST)
Malondialdehyde……………………………………………………………… (MDA)
enzyme-linked immunosorbent assay……………………………………….. (ELISA)
Horseradish Peroxidase ………………………………………………………(HRP)
Glutathione ……………………………………………………………………(GSH)

INTRODUCTION AND LITERATURE REVIEW

1. INTRODUCTION

The oral cavity is the only place in the body which is exposed to numerous environmental factors. These include air pollution, tobacco smoke, food, medicines, and microorganisms. Most of them increase the production of reactive oxygen species (ROS), which has a destructive effect not only on redox homeostasis of the oral cavity but also of the whole body (Żukowski et al., 2018).

It is not surprising that saliva has specialized antioxidant mechanisms to protect against overproduction of ROS in the oral cavity. Thus, saliva is the first line of defense against oxidative stress . Enzymatic antioxidants, for example, superoxide dismutase (SOD, E.C. 1.15.1.1), glutathione peroxidase (Px, E.C. 1.11.1.9), glutathione reductase (E.C. 1.6.4.2), and catalase (CAT, E.C. 1.11.1). play a key role in this process. They have a preventive function, preventing ROS and its derivatives from reacting with biological particles. They form an intracellular antioxidant system that regulates i.a. the gene transcription process or the inflammatory response. Extracellular activity is the enzymatic degradation of ROS in biological fluids. SOD catalyzes the dismutation (disproportionation), that is a reaction in which one superoxide anion radical converts to oxygen and hydrogen peroxide. SOD also shows enzymatic activity in relation to other substrates such as singlet oxygen. Px catalyzes the reduction of hydrogen peroxide (H2O2) and organic peroxides with reduced glutathione (GSH), and, by oxidation of nicotinamide adenine dinucleotide phosphate (NADPH), it reproduces the reduced glutathione form (Wang et al., 2017).

CAT shows two activities depending on the hydrogen peroxide concentration in a cell. At high H2O2 concentration, this enzyme catalyzes the dismutation of

hydrogen peroxide, which leads to the production of oxygen that is used by the body in metabolic processes. At low concentrations of hydrogen peroxide, this enzyme acts as peroxidase. Peroxidase activity is mainly related to H2O2 deactivation. Periodontitis is initiated by a bacterial infection and then progresses as a result of an abnormal immune response of a host, contributing to the formation of dysbiotic subgingival biofilm and the progressive destruction of periodontal tissues. It may be related to systemic oxidative stress (Liu et al., 2014).

Periodontitis is a prevalent inflammatory disease, influencing at least 10% of people worldwide. It can result in the destruction of teeth supporting tissue and ends up with a loss of teeth. In addition, periodontitis has been suggested to have moderate association with several systemic diseases, e.g., cardiovascular disease, diabetes, and adverse pregnancy outcomes. Two principal forms of periodontitis are currently recognized, chronic (CP), and aggressive periodontitis (AgP). AgP is a specific form of periodontal disease with a higher rate of progression and patterns of tissue destruction, mostly affecting younger individuals. (Nazir, 2017) Despite differences in their clinical phenotypes, no unequivocal pathophysiological foundation that differentiates between CP and AgP has been established. Chronic and aggressive periodontitis lesions cannot be distinguished on the basis of histopathologic features or microbial colonization profiles, although there is evidence of immunologica differences, including the presence of neutrophil abnormalities in AgP. Neutrophils play a central role in the initial host inflammatory response to the periodontal pathogens and protect the host tissues by killing various pathogenic bacteria either by non-oxidative or oxidative means, in an intracellular or extracellular environment. Non-oxidative killing is mediated by various lysosomal enzymes, peptides and proteins, including lysozyme, bactericidal/permeability- increasing proteins, cationic proteins, defensins, and lactoferrin. Generation of ROS (superoxide, hydrogen peroxide,

hydroxyl radicals, hypochlorous acid, and chloramines) contributes to the oxidative killing of the invading microorganisms. Unfortunately, the ROS generated during an oxidative burst response can cause considerable collateral damage and are directly responsible for infection associated tissues injuries. Neutrophils from patients with AgP are hyperactive and primed and appear to release enhanced levels of oxygen radicals, inflammatory mediators such as cytokines and matrix-degrading enzymes (Trivedi and Lal, 2017). This hyperactivety and reactivity of neutrophils destroys the adjacent host tissues and contributes to the destructive changes observed in inflammatory periodontitis. An excess of ROS can cause oxidative stress and damage to critical biomolecules resulting in deleterious biological effects. Concept suggests that this inflammatory disease is initiated by bacterial infection and subsequently progressed by aberrant host response, which mainly contributes to periodontal tissue destruction. In recent years, reactive oxygen species (ROS) have gained more and more attention, because of their central role to the progression of many inflammatory diseases. ROS are described as oxygen free radicals and other non-radical oxygen derivatives involved in oxygen radical production. They are involved in normal cellular metabolism and continuously generated by the cells in most tissues. Another category of substances called antioxidants exist in the cells and can effectively delay or inhibit ROS-induced oxidation. Under physiological conditions, ROS are effectively neutralized by antioxidants, which prevent ROS-mediated tissue damage. When inflammation happens, ROS production is drastically increased mainly due to cells of innate immune system, e.g., neutrophils and macrophages during Wang et al. Oxidative Stress and Periodontitis the process of phagocytosis via the metabolic pathway of the "respiratory burst". Subsequently, high levels or activities of ROS cannot be balanced by the antioxidant defense system, which leads to the oxidative stress and tissue damage . ROS can directly cause tissue damage, involving lipid

peroxidation, DNA damage, protein damage, and oxidation of important enzymes; meanwhile, they can function as signaling molecules or mediators of inflammation (Lushchak, 2014). Over the past few years, numerous clinical and basic experimental studies have shown a strong association between oxidative stress and periodontitis. Getting a better understanding of this association can give us a deeper insight periodontitis and systemic inflammation, and therapeutic strategies. Chronic and aggressive periodontitis are characterized by an increased production of reactive oxygen species ROS, such as superoxide anion, hydroxyl radical, and peroxyl radical, can damage many biological molecules including DNA, lipids, and proteins. Prolonged existence of these ROS promotes severe tissue damage and cell death. Malondialdehyde (MDA) is the principal product of polyunsaturated fatty acid peroxidation that can indicate the increase of oxidative stress. It's well known that inflammation is strongly associated to increased oxidative stress components in periodontal disease. Thus, an uncontrolled inflammatory response in periodontitis can serve as an intermediate variable between them. Periodontitis patients exhibit changes in local and systemic antioxidant and oxidative stresses. Saliva as a mirror of body health may reflect general health and may be used as a diagnostic material in future (Rizwan et al., 2014).

1.1. Periodontitis

Periodontitis is a disease that affects dental support structures. Periodontitis can be divided in three main forms: chronic, aggressive and as a manifestation of a systemic disease. Chronic periodontitis (CP) is prevalent form of this disease and generalized form involves more than 30% of dentition . Periodontitis is an inflammatory condition in which the infiltration of mononuclear cells into the gingival tissue results in connective tissue and alveolar bone destruction.

Although bacteria are causes of periodontitis, development and severity of disease are characterized by the host immune reaction. The exact mechanism of periodontal tissue destruction has not yet been clarified. Immunohisto chemical studies have shown that infiltration of mononuclear inflammatory cells below the basal layer of periodontal pockets increase the pathogenesis stems from a combination of factors, involving periodontal bacteria, inflammatory immune responses, patient behavioral (life style) and concomitant medical conditions. More recently this pathology has been linked to several metabolic diseases, including diabetes and cardio vascular diseases. The association between aging and periodontitis is not conclusively proved. Recently, it has been suggested that the increase of inflammation accompanying periodontitis is considered as a risk factor for certain systemic diseases such as diabetes type 2 cardiovascular and cerebrovascular diseases (Reddy, 2017).

1.2. Periodontal diseases classification

Plaque-induced Gingivitis: An inflammation of the gingiva induced by bacteria located at the mgingival margin. Chronic Periodontitis: Inflammatory disease affecting the supporting tissues of the teeth, characterized by progressive loss of tooth attachment to periodontal tissues. It is associated with periodontal pocket formation and/or gingival recession. Both localized and generalized disease forms can occur.

Aggressive Periodontitis: A rare form of inflammatory periodontal disease, characterized with by rapid disease progression in patients who present with no common etiologies and risk factors for periodontal disease. Both localized and generalized disease forms can occur.

Refractory Periodontitis: A group of destructive inflammatory periodontal diseases affecting patients who do not respond to conventional periodontal

therapy, and present with continued attachment loss over time, despite well-controlled known risk factors for disease progression.

1.2.1. Plaque-induced Gingivitis:

Plaque-induced gingivitis is defined as an inflammation of the gingiva induced by bacteria located at the gingival margin. The causative relationship between bacterial plaque (biofilm) and gingival inflammation was demonstrated in an experimental gingivitis study. Characteristic gingivitis clinical signs include erythema, edema, loss of gingival stippling and bleeding on probing (BOP). Interestingly, the host response to similar plaque levels varies significantly among patients. Histologic changes in the tissue include proliferation of junctional epithelium, vasculitis of blood vessels adjacent to the junctional epithelium, collagen degradation, cytopathologic alteration of fibroblasts, and inflammatory infiltrate. Gingivitis is reversible upon removal of the etiologic biofilm (Tatakis and Trombelli, 2004).

1.2.2. Chronic Periodontitis:

Similar to gingivitis, CP is also an inflammatory disease caused by the bacterial biofilm. In fact, gingivitis is an established risk factor for CP (Lang et al., 2009). CP commonly develops in the 4th Plaque-induced Gingivitis: An inflammation of the gingiva induced by bacteria located at the gingival margin. Chronic Periodontitis: Inflammatory disease affecting the supporting tissues of the teeth, characterized by progressive loss of tooth attachment to periodontal tissues. It is associated with periodontal pocket formation and/or gingival recession. Both localized and generalized disease forms can occur. Aggressive Periodontitis: A rare form of inflammatory periodontal disease, characterized with by rapid disease progression in patients who present with no common etiologies and

risk factors for periodontal disease. Both localized and generalized disease forms can occur. Refractory Periodontitis: A group of destructive inflammatory periodontal diseases affecting patients who do not respond to conventional periodontal therapy, and present with continued attachment loss over time, despite well-controlled known risk factors for disease progression. decade of life, with highest prevalence in senior individuals (Eke et al., 2012).

CP is characterized by extension of gingival inflammation to the alveolar bone, connective tissue degradation and net loss of tooth attachment to periodontium. The disease is further defined by its extent (localized if 30% of sites are affected), and by its severity, as measured by CAL (mild: 1-2mm CAL; moderate 3-4mm CAL; severe >5mm CAL). As opposed to gingivitis, which is not associated with specific pathognomonic bacteria, several bacterial species are considered periodontogenic. The members of the red complex - Porphyromonas gingivalis, Tannerella forsythia, and Treponema denticola were consistently demonstrated to be elevated in CP patients compared to periodontally-healthy subjects, and in diseased sites compared to healthy sites of diseased subjects . Conventional periodontal therapy for CP includes scaling and root planing that focus on the removal of etiologic biofilms and calculus. In most cases this form of therapy allows for resolution of inflammation to occur and for periodontal tissues to heal. Surgical re-establishment of maintainable periodontal tissues generally follows scaling and root planing in severe cases of CP (Genco and Van Dyke, 2010).

If left untreated, CP results in pain, discomfort, tooth mobility, and eventually – tooth loss, and may represent a risk factor for multiple systemic conditions with underlying low grade inflammation such as metabolic syndrome, diabetes and cardiovascular disease. Clinically, there is great diversity in patient responses to the bacterial biofilm. CP patients may differ in the rate of disease progression, severity of periodontal tissue destruction, and treatment outcomes. Furthermore, clinical studies have demonstrated that in some individual’s gingivitis never progresses to CP, regardless of periodontal care. Although certain pathogenic bacteria in subgingival biofilms produce specific virulence factors that

could cause direct damage to periodontal tissues, current evidence suggest that it is the host factors that drive periodontal tissue degradation at sites with CP. These factors include over-expression of inflammatory cytokines, proteolytic enzymes, and increased oxidative stress. The rate limiting steps in onset and progression of clinical attachment loss are incompletely understood. Increasing evidence that emerged in recent years indicates that failure to resolve biofilminduced periodontal inflammation results in chronicity and pro-osteolytic environments (Van Dyke, 2014).

1.2.3. Aggressive Periodontitis:

Aggressive periodontitis (AgP) is comprised of a group of rare, often severe, rapidly progressing forms of periodontitis affecting up to 5% of population depending primarily on race/ethnicity. AgP has a distinctive familial pattern, and patients generally have noncontributory medical history. Unlike CP, AgP is more likely to be diagnosed in younger individuals (<30 years of age), although age per-se is no longer considered a diagnostic criteria. Similar to CP, AgP occurs in localized and generalized forms. The localized form most commonly affects permanent first molars and incisors, while the generalized form is characterized by generalized interproximal attachment loss affecting at least 3 permanent teeth other than the first molars and incisors. Common clinical features may include bacterial biofilm levels that are inconsistent with the severity of periodontal tissue destruction, elevated proportions of the periodontal pathogen Aggregatibacter actinomycetemcomitans (AA), and neutrophil function abnormalities. Antibiotic treatment in conjunction with scaling and root planing is recommended for AgP, as specific bacterial etiology has been demonstrated. Altered neutrophil function is a common finding among AgP patients, suggesting a key role of altered host responses in periodontitis severity and rate of progression. This role is further supported by the observation that disease progression may be self-arrested. AgP is treated in a similar manner to CP with more emphasis on the host than on the biofilm. The use of immune-modulatory therapeutics and antimicrobials are used more often than for CP with varying

degrees of success. In some instancespatients with AgP or CP are refractory to conventional periodontal therapy and may be diagnosed with refractory AgP or refractory CP, respectively (Susin et al., 2014).

1.2.4. Refractory Periodontitis:

Refractory patients have a recurring disease progression pattern and continuous loss of clinical attachment after treatment, which does not correlate with plaque levels, microbiology assessments, and treatment compliance (Fardal and Linden, 2008).

Therefore, patients with either CP or AgP are diagnosed with refractory disease only after active periodontal treatment, periodontal maintenance and follow-up . Refractory patients were demonstrated to lose a significantly higher number of teeth during periodontal maintenance when compared to non-refractory patients Man. agement of disease in refractory patients consists of long-term subantimicrobial dose doxycycline (SDD) in conjunction with routine periodontal maintenance. SDD was demonstrated to inhibit matrix metalloproteinase activity, thus preventing connective tissue destruction. A nine-month longitudinal study demonstrated significantly greater mean CAL and mean PD reductions in patients receiving daily SDD when compared to placebo treatment (Preshaw et al., 2008).

1.3. Epidemiology of Periodontitis:

Periodontitis is widely regarded as the second most common disease worldwide, after dental decay, and in the United States has a prevalence of 30–50% of the population, but only about 10% have severe forms. Like other conditions that are intimately related to access to hygiene and basic medical monitoring and care. Periodontitis tends to be more common in economically disadvantaged populations or regions. Susin and associates reported that periodontitis is a major concern in Brazil with prevalence as high as 79% depending on the adopted diagnostic criteria. Generally, in Yemenite, North-African,

South Asian, or Mediterranean origin have higher prevalence of periodontal disease than individuals from European descent individuals living in 0TEast Asia0T (e.g., Japan, South Korea and Taiwan) have the lowest incident of periodontal disease in the world. This could be attributed to genetic predisposition as well as cultural-behavioral differences (e.g, smoking, oral hygiene, and access to dental treatment) between populations (Zadik et al., 2008).

1.4. Risk factors of Periodontitis:

Periodontitis presents a multifactorial etiology which makes its study complex. There are some denominators such as stress, smoking, DM, older age, osteoporosis, genetic predisposition, low educational or socioeconomic conditions, and the presence of infections with certain bacteria and viruses. Genetic polymorphisms represent an important component in susceptibility to periodontitis. Moreover, accumulating evidence demonstrates that genetic variations in genes that codify for proinflammatory cytokines could affect the systemic inflammatory response in periodontitis patients (Lim et al., 2007).

1.5. Pathogenesis of Periodontitis:

The development of periodontitis is related with a specific group of predominantly Gram-negative, anaerobic or microaerophilic bacteria. They colonize on the root surface in the subgingival area. The key periodontal pathogens in chronic periodontitis are (Porphyromonas gingivalis, Tannerella forsythia and Treponema denticola). whereas those in localized aggressive periodontitis are Aggregatibacter (formerly termed Actinobacillus) actinomycetemcomitans. Although bacteria are essential, the bacteria alone are not sufficient for the disease to occur. It was well- recognized that host responses to the periodontal pathogens and their virulence factors play an important role in the pathogenesis of periodontitis. (Jain and Darveau, 2010) After colonization on the gingival sulcus by periodontal bacteria, the bacteria release their products, for example,

lipopolysaccharide (LPS) which is well known virulence factor of Gram -negative bacteria. LPS has the potential to interact with nearly all cell types in periodontal tissues including immune cells and resident cells and thereby initiating a defense mechanism. The initial immune response in periodontal disease is characterized by the action of the innate immune system which, in this context, consists of the gingival epithelium, fibroblasts, neutrophils, dendritic cells, and monocytes/macrophages. In fact, innate host recognition of LPS is a key initiating event for the subsequent clearance of Gram-negative bacteria from infected host tissues. A group of receptors called pattern recognition receptors (PRRs) which include cell surface Toll-like receptors (TLRs) and intracellular NO-like receptors (NLRs) are responsible for the detection of microbial-associated molecular patterns (MAMPs), i.e., LPS, and thereby leading to cellular activation (Pathirana et al., 2010). However; little knowledge is yet known how NLRs sense oral bacteria. In the gingival epithelium, the binding of LPS to TLR of nearby cells induces the production of cytokines and chemokines resulting in the expression of adhesion molecules, increased permeability of gingival capillaries and chemotaxis of polymorphonuclear neutrophils (PMNs) through the junctional epithelium and into the gingival sulcus to phagocyte bacteria. One important component of innate immunity that plays a vital role in periodontal disease is monocytes. In response to inflammatory signals, monocytes can migrate quickly to sites of infection in the tissues and differentiate into macrophages which can effectively capture invading pathogens. The phagocytosis of bacteria by macrophages results in cytokine secretion and antigen-presentation to induce a more effective adaptive immunity. Later on, if the plaque biofilm matures further, the pathogenic species developing in the periodontal pockets release an array of virulence factors, antigens or by products particularly LPS, into the pocket junctional epithelium, blood vessels and deeper connective tissues of periodontium. This leads to a chronic inflammatory response characterized by dysregulation of immune-bacteria interactions where the infected tissues/cells are overwhelmed by the persistent pathogens accompanied with continuous and excessive production of potent proinflammatory

cytokines (i.e. IL-1β, TNF-α, IFN-γ and IL-6) . Consequently, as the disease progresses to the more advanced stage(s), the specific cytokines and chemokines produced by innate immune response direct the host response towards a robust cell-mediated adaptive immunity (Liu et al., 2014). The dominant perivascular T-cell/macrophage infiltrate is observed in the connective tissues If this T-cell response does not overcome the bacterial challenge, the disease proceeds to B-cell/plasma-cell dominated lesion. The production of antibodies by B-cell/plasma may be protective and control the infection. The characteristics of chronic periodontitis is mediated by the Bcell/plasma cell response, the immunohistological features of chronic periodontitis are characterized by an apical migration of plaque on the root surface, accompanied by subgingival calculus formation. At this stage, a predominance of plasma cell infiltrates with few macrophages is observed in connective tissue. There is an alteration in appearance and reduction in the number of local fibroblasts with the formation of the encapsulated fibrous band surrounding the body of the lesion. High levels of IL-1 and IL-6 produced from infiltrating cells lead to the production of matrix metalloproteinases (MMPs), especially by fibroblasts which, in turn, results in further attachment loss and bone resorption (Smith et al., 2010). Clearly, periodontal disease is a multi-factorial disorder. The primary cause of periodontal disease is plaque bacteria but the disease progression is modified by an individual's susceptibility. A wide variety of determinants and factors, either environmental or acquired, e.g. smoking, diabetes, systemic diseases, genetic factors, microbial composition of dental plaque are known to influence the host response. Therefore, these factors could subsequently have certain effects on the disease initiation and progression. So that, a complete understanding how diabetes contributes periodontal disease progression could be a key to establish rational diagnostic and therapeutic strategies for periodontal disease associated with diabetes (Anner et al., 2010).

1.6. Oxidative stress:

Organic free radicals were first discovered (in vitro) by Moses Gomberg in 1900. He did not find them to be involved in any biological processes. Later, it was not until much later in 1950s, free radicals were implicated in aging and in pathological processes. Since that time, research has been focused on investigating the different roles and implications of free radicals in health and disease and the biologic mechanisms to regulate them. Free radicals are described as atoms or groups of atoms with unpaired electrons. They are very unstable and react quickly with other compounds, trying to capture the needed electron to gain stability. Once these highly reactive radicals are formed, they can start a chain reaction that can result in the disruption of a living cell. Some free radicals are necessary in different biochemical reactions, however if they are found in excess, they can be highly toxic and react with a wide variety of biological molecules including lipids, proteins, carbohydrates, and DNA.There are different kinds of radicals, but one of the most common and important are the ones derived from molecular oxygen, called reactive oxygen species (ROS). These molecules have the potential to cause a number of damaging events. The oxygen molecule in the ground state is a bi-radical, containing two unpaired electrons in separate orbitals in its outer electron shell. This is an extremely unstable configuration, and radicals quickly react with other molecules to achieve stability. Sequential reduction of molecular oxygen leads to formation of a group of ROS including superoxide anion, peroxide (hydrogen peroxide), hydroxyl radical, and singlet oxygen (Lushchak, 2014).

1.6.1. Formation of ROS:

There are different sources of oxygen radicals and ROS formation. It was originally thought that only phagocytic cells were responsible for ROS production as they take part in host cell defense mechanisms against bacterial pathogens. To date, many sources of ROS have been discovered. They can be classified as exogenous or endogenous sourced Figure(1-1). Exogenous sources in the oral cavity include heat, trauma, ultrasound,

ultraviolet light, air pollution, smoking, exhaust fumes, chemicals, toxins, pathogenic microorganism, radiation, infection, excessive exercise, therapeutic drugs, fried foods, etc. Endogenous sources are as a result of formation in the mitochondria (as oxygen is reduced along the electron transport chain), phagocytes, cytochrome P-450 reactions, inflammation, etc. (Srejovic et al., 2013) It has been established that in eukaryotic cells over 90% of ROS are produced by mitochondria. Mitochondrial adenosine triphosphate (ATP) production is the main energy source for intracellular metabolic pathways. The process, in which ATP is produced, called oxidative phosphorylation, involves the transport of protons (hydrogen ions) across the inner mitochondrial membrane by means of the electron transport chain (ETC). In the ETC, electrons are passed through different carries. These carriers are specific; each one accepts electrons from a specific type of carrier or its predecessor. Electrons pass from complex I to Conezyme Q, and from Coenzyme Q to complex III. From Complex III the pathway is to cytochrome c then to a Complex IV. It is at this site that oxygen binds, and using the electron pair and remaining free energy, oxygen is reduced to produce water. However, in about 0.1–2% of electrons passing through the chain (this percentage is related to studies in isolated mitochondria) escape the ETC and interact with molecular oxygen to give the superoxide radical (O-2). Superoxide is not extremely reactive by itself, but can inactivate specific enzymes or initiate lipid peroxidation in its protonated form, hydroperoxyl HO2. Furthermore, there are minor amounts of ROS that are produced by ETC located at the endoplasmatic reticulum (ER), plasmatic, and nuclear membranes as well as some oxidases. The production in the ER is through the cytochrome P450 family enzymes, and the production of superoxide occurs similarly to that of the mitochondria (Skulachev, 2012).

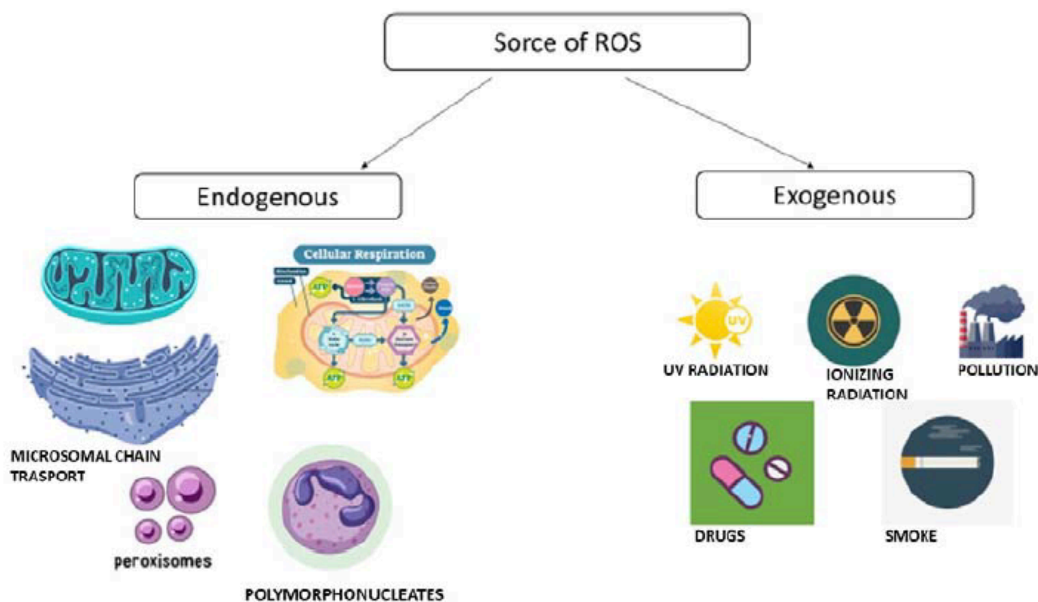


Figure 1. These are the main source of ROS (reactive oxygen species). (Skulachev, 2012)

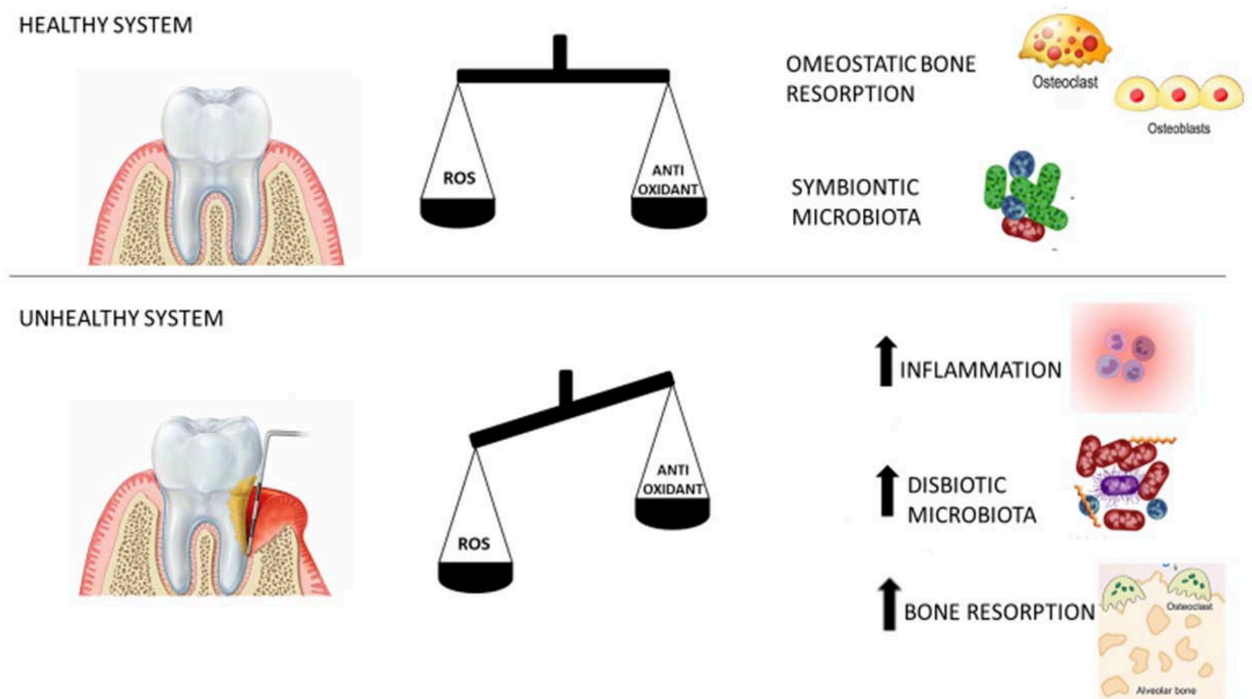
1.6.2. Biological effects of Reactive Oxygen Species:

Recent work has demonstrated that ROS plays a dual role as both damaging and beneficial species, since they can be either harmful or beneficial to living systems. Low levels of ROS are generated in a number of reactions essential to life. They have a role in cell signaling, including; growth arrest and apoptosis; gene expression; cellular differentiation; and the activation of cell signaling cascades. It should be noted that ROS can serve as both intra- and intercellular messengers (Roberts and Sindhu, 2009)In normal physiology there is a dynamic equilibrium between ROS activity and antioxidant defense capacity. When that balance shifts in favor of ROS, either by a deficiency of enzymatic and non-enzymatic antioxidant defenses or an increase in ROS production or activity, reactive oxidative stress occurs. An excess of ROS can cause result in damage of cellular lipids, proteins, or DNA inhibiting their normal function. This delicate equilibrium between favorable and destructive effects of free radicals is an essential aspect of living organisms and is accomplished by mechanisms called “redox regulation”. Redox reactions, or oxidation-reduction reactions, are a family of reactions that are implicated with the exchange of electrons between species. Oxidation refers to the loss of electrons, while reduction refers to the gain of electrons. The process of “redox regulation” protects

the body from various oxidative stresses and maintains “redox homeostasis” in the living organism. In oxidative stress, the redox balance between oxidants and antioxidants is shifted towards oxidative potential. Different oxidative stress markers include, malondialdehyde (MDA), isoprostanes, 8- hydroxydeoxyguanosine (8-OHDG). etcThe elimination of RS by antioxidant defense systems is indispensible for conserving health. Anti-oxidants are compounds, which will inhibit the oxidation of other molecules while providing electrons to free radicals to neutralize them. The compounds are able to accommodate the loss of an electron without becoming reactive. Some examples of these anti-oxidants are beta-carotene, Vitamin A, Vitamin E, Vitamin C, and glutathione peroxidase, etc (Almerich-Silla et al., 2015).

1.7. Role of Oxidative Stress in Periodontal Disease:

ROS has many roles in the periodontal tissue. During the correct equilibrium of oxidative stress, ROS behave as the cellular messenger, stimulate the production of molecules for the correct function of the cells, and stimulate the immune system to react against pathogens. The presence of pathogens in the periodontal pocket stimulates the release of cytokines, which recall polymorphonucleates to phagocyte the invaders. During the macrophage’s activity, they produce free radicals causing an increase in the concentration of ROS. The ROS level increases in a healthy system allowing the killing of pathogens and promoting the response of the host immune system. In this case, ROS perform a protective role. Unfortunately, when the infection persists, the balance is broken, and the ROS increase becomes a cause of illness Figure (1-2) (Matthews et al., 2007).



Figure(1- 2). In a healthy system there is a balance between reactive oxygen species (ROS) and antioxidant This equilibrium allows for structures. If ROS level, for some reason, becomes higher than the antioxidant level, the balance isbroken. In that situation, the high level of ROS causes inflammation in the periodontal tissue. (Ling et al., 2016).

In this research, the presence of ROMs (reactive oxygen metabolites) in the serum was evaluated. As a result, these studies showed that the ROM level was higher in the periodontopathic patients compared to the healthy ones.

The resorption of the alveolar bone present in periodontitis is based on the alteration of the homeostatic axis. Normally, there is a homeostatic axis that has the role to stimulate Figure 2. In a healthy system there is a balance between reactive oxygen species (ROS) and antioxidant concentration. This equilibrium allows for maintaining homeostasis in the periodontal tissue If broken. In that situation, the high level of ROS causes inflammation in the periodontal tissue. At the same time, the microbiota communities change in favor of virulent bacterial communities, this causes the instauration of a dysbiotic microbiota. If this condition is not resolved, it becomes chronic, and the permanence of these mechanisms causes the destruction of the periodontal tissues.

In different studies, the relationship between periodontitis and ROS was investigated. In this research, the presence of ROMs (reactive oxygen metabolites) in the serum was evaluated. As a result, these studies showed that the ROM level was higher in the periodontopathic patients compared to the healthy ones (Ling et al., 2016).
The resorption of the alveolar bone present in periodontitis is based on the alteration of the homeostatic axis. Normally, there is a homeostatic axis that has the role to stimulate the neo-apposition of bone and the resorption of the old one. In periodontitis, the increase in ROS in the periodontal pockets causes the overproduction of cytokines that break the RANKL/osteoprotegerin axis. When this equilibrium is altered, we have inflammatory bone-related illnesses such as periodontitis, rheumatoid arthritis, osteoarthritis, and osteoporosis.
As mentioned before, periodontitis leads to soft tissue distraction. Studies showed that one of the causes of clinical attachment loss is the activation of the metalloproteases (MMPs) caused by ROS oxidation, for instance, hydrogen peroxide (Franco et al., 2017). It was seen that when there is an oxidative stress environment, different elements of the periodontium such as collagen, elastin, proteoglycans, and glycosaminoglycans (hyaluronic acid) started to be degraded. Therefore, the oxidated fatty acids trigger adipogenesis and inhibit osteogenesis, causing the periodontium components' degradation It was documented that ROS reduces the production of collagen in the cells present in the extracellular matrix (Nessa et al., 2021).
The altered concentration of ROS and the material produced by the destruction of periodontal tissue are substrates that stimulate the release of cytokines and the immune system. In this way, it establishes a circuit that feeds the persistence of chronic inflammation.
Moreover, in the presence of risk factors, the ROS concentration is higher and exaggerates periodontal disease. Risk factors such as smoking cigarettes, diabetes, and cardiovascular disease have in common the production of ROS and the enhancement of oxidation reactions with periodontitis. Tobacco is an exogenous origin of ROS(Rapone et al., 2022).

In diabetic patients, the resistance to insulin causes a decrease in antioxidants and, as a response to the system, ROS concentration increases . In cardiovascular disease, it was seen an association with periodontitis. The two pathologies have the same risk factors in common; both present an alteration of oxidative stress and inflammation . Furthermore, a new study reported that the type of diet followed can influence the inflammation of the soft tissue around the tooth. This is because diet impacts the oral biofilm in favor of a healthy or an unhealthy environment(Schenkein et al., 2020).

Materials and Methods

2.1. Experimental design

In this study 85 samples (were included with age range (30-50) years. Two groups of samples were included in this study. Group (1) contained people with periodontitis (n=45) (23 males and 22 females), they were compared to a apparently healthy individual Group (2) (n=40) (20 males and 20 females) control who matched in age and gender. Patients were clinically diagnosed with periodontitis. The samples were collected from Dentists clinics in Erbil City. Patients were assessed by full medical history in order to exclude any existing systemic disease.

2.2. Collection of blood samples

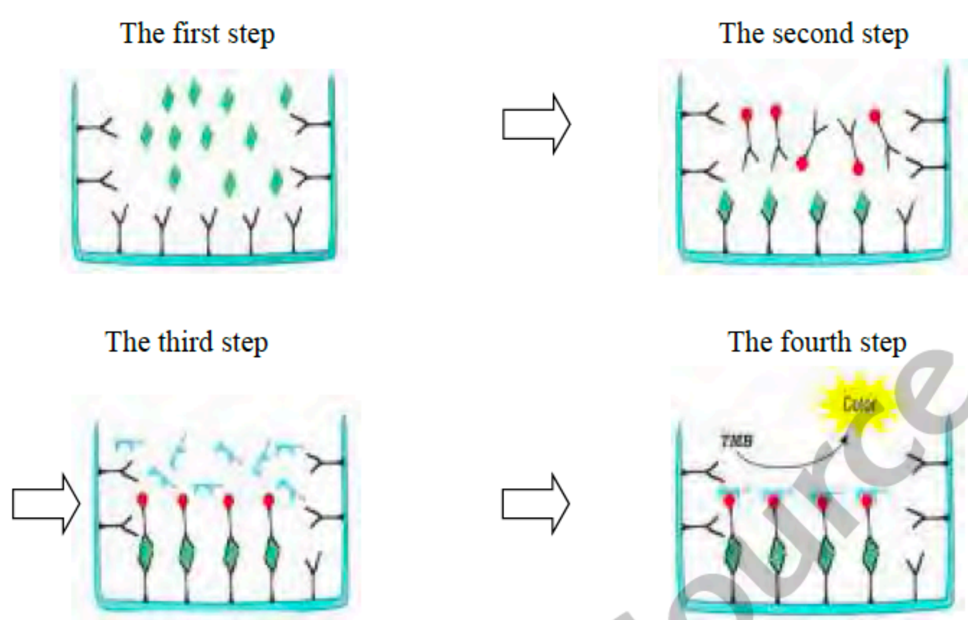
About 5mL of venous blood was taken from each individual, collected in gold-top serum separator tubes (SST), allowed standing for 15 minutes, separation of serum from blood cell was performed by centrifugation at (3000 rpm) for 15 minutes. The obtained serum was transferred immediately to pre-labeled and coded Eppendorf tubes. These samples were frozen at –20°C for upcoming investigation.

2.3. Determination of human Malondialdehyde (MDA) concentration

The concentration of total **MDA** (Malondialdehyde)) in serum samples was determined by sandwich enzyme- linked immunosorbent assay (ELISA) technique using the kit manufactured by BioVision company

Principle of the assay

This ELISA kit uses Competitive-ELISA as the method. The microtiter plate provided in this kit has been pre-coated with the antigen. During the reaction, the antigen in the sample or standard competes with a fixed amount of the antigen on the solid phase suppporter for sites on the Biotinylated Detection Ab specific to the antigen. Excess conjugate and unbound sample or standard are washed from the plate, and Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. Then a TMB substrate solution is added to each well. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution



and the color change is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The concentration of the antigen in the samples is then determined by comparing the O.D. of the samples to the standard curve.

Assay procedure:

1. Add Sample and Biotinylated Detection Ab: Add 50μl of Standard, Blank, or Sample per well. The blank well is added with sample diluent. Immediately add 50 μl of Biotinylated Detection Ab working solution to each well. Cover with the Plate sealer we provided. Gently tap the plate to ensure thorough mixing. Incubate for 45minutes at 37°C. (Solutions are added to the bottom of micro-ELISA plate well, avoid inside wall touching and foaming as possible.)

2. Wash: Aspirate each well and wash, repeating the process three times Wash by filling each well with Wash Buffer (approximately 350μl) using a squirt bottle, multi-channel pipette, manifold dispenser or automated washer. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and pat it against thick clean absorbent paper.

3. HRP Conjugate: Add 100μl of HRP Conjugate working solution to each well. Cover with a new Plate sealer. Incubate for 30 minutes at 37°C.

4. Wash: Repeat the aspiration/wash process for five times as conducted in step 4.

5. Substrate: Add 90μl of Substrate Solution to each well. Cover with a new Plate sealer. Incubate for about 15 minutes at 37°C. Protect from light. The reaction time can be shortened or extended according to the actual color change, but not more than 30 minutes. When apparent gradient appeared in standard wells, you can terminate the reaction.

6. Stop: Add 50μl of Stop Solution to each well. Color turn to yellow immediately. The adding order of stop solution should be as the same as the substrate solution.

7. OD Measurement: Determine the optical density (OD value) of each well at once, using a microplate reader set to 450 nm. You should open the microplate reader ahead, preheat the instrument, and set the testing parameters. 8. After experiment, put all the unused reagents back into the refrigerator according to the specified storage temperature respectively until their expiry. Average the duplicate readings for each standard and samples. Create a standard curve by plotting the mean OD value for each standard on the

y-axis against the concentration on the xaxis and draw a best fit curve through the points on the graph. It is recommended to use some professional software to do this calculation, such as curve expert 1.3 or 1.4. In the software interface, a best fitting equation of standard curve will be calculated using OD values and concentrations of standard sample. The software will calculate the concentration of samples after entering the OD value of samples.

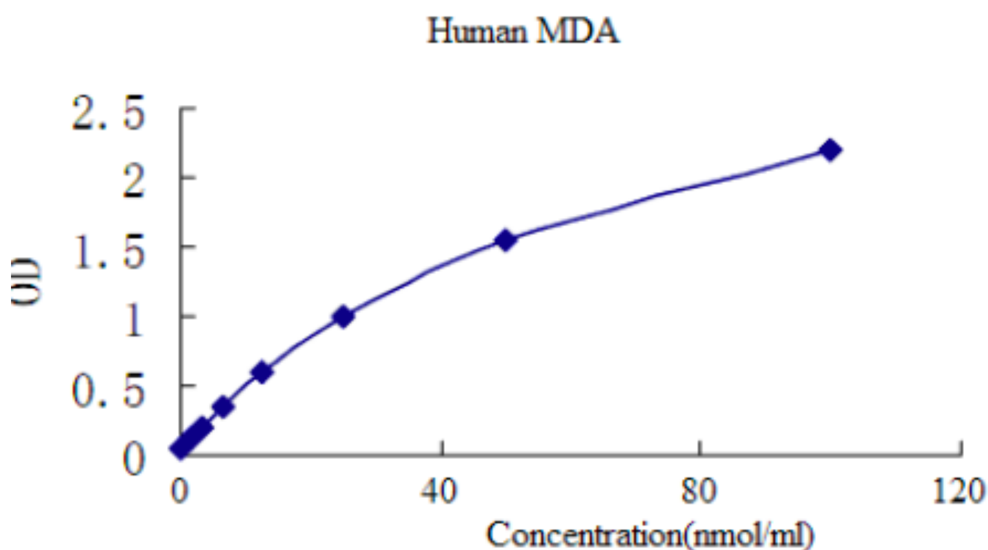


Figure 2-1: Total MDA calibration curve

2.4. Determination of human superoxidase dismutase (SOD) concentration

The concentration of total SOD in serum samples was determined by sandwich enzyme-linked immunosorbent assay (ELISA) technique using the kit manufactured by BioVision company.

principle

Human Superoxide dismutase (SOD) ELISA Kit employs a two-site sandwich ELISA to quantitate SOD in samples. An antibody specific for Human SOD has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any SOD present is bound by the immobilized antibody. After removing any unbound substances, HRP-Conjugated SOD detection antibody is added to the wells. Following a wash to remove any unbound HRP reagent, a Chromogen solution is added to the wells and color develops in

proportion to the amount of SOD bound in the initial step. The color development is stopped and the intensity of the color is measured.

Assay procedure

1. Prepare all reagents before starting assay procedure. It is recommended that all Standards and Samples be added in duplicate to the microplate.

2. Add standard: Set Standard wells, testing sample wells. Add diluted standard 50 μL to standard well.

3. Add Sample: Add sample diluent 40 μL to testing sample well. Then add sample 10 μL to testing sample well, Blank well doesn't add anything.

4. Cover with a plate cover and incubate for 45 minutes at 37 °C.

5.Aspirate each well and wash, repeating the process four times for a total of five washes, 1-3 minutes per time. Wash by filling each well with Wash buffer (250 μL) using a squirt bottle, manifold dispenser or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

6. Add HRP-Conjugated detection antibody 50 μL to each well, except blank well.

7. Cover with plate cover. Incubate for 30 minutes at 37 °C.

8. Repeat the aspiration/wash process for five times as in step 5.

9. Add chromogen solution A 50 μL and chromogen solution B 50 μL to each well. Gently mix and incubate for 15 minutes at 37°C. Protect from light.

10. Add 50 μL Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.

11. Read the Optical Density (O.D.) at 450 nm using a microtiter plate reader within 15 minutes.

CALCULATION OF RESULTS

Known concentrations of SOD standards and their corresponding OD readings were plotted on the scale (x-axis) and scale (y-axis) respectively. GraphPad Prism and MS Excel computer software were used for data plotting and finding the best curve fitting method. The concentration of human total SODs in samples were determined by plotting their OD values on the Y-axis of the calibration curve. The original concentration was calculated by multiplying it with the dilution factor.

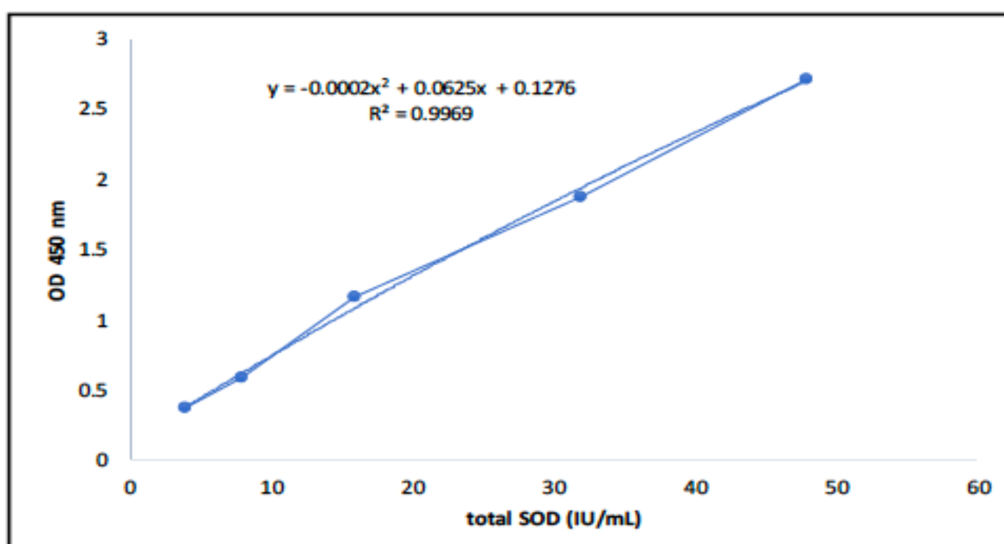


Figure 2-2: Total SOD calibration curve

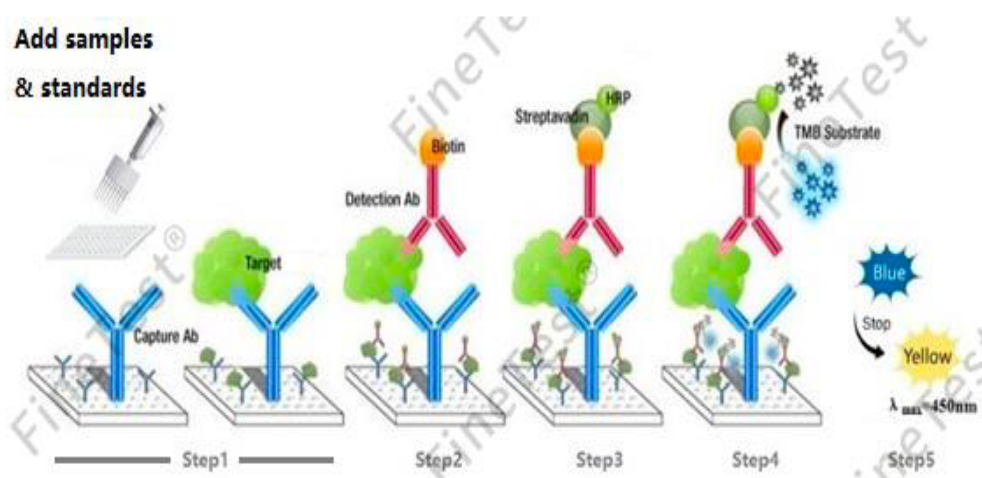
2.5. Determination of Human Glutathione (GSH) concentration

The concentration of total GSH in serum samples was determined by sandwich enzyme-linked immunosorbent assay (ELISA) technique using the kit manufactured by BioVision company.

Principle of the assay

This kit was based on sandwich enzyme-linked immune-sorbent assay technology. Capture antibody was pre-coated onto 96-well plates. And the biotin conjugated antibody was used as detection antibodies. The standards, test samples and biotin conjugated detection

antibody were added to the wells subsequently, and washed with wash buffer. HRP-Streptavidin was added and unbound conjugates were washed away with wash buffer. TMB substrates were used to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The density of yellow is proportional to the target amount of sample captured in plate. Read the O.D. absorbance at 450nm in a microplate reader, and then the concentration of target can be calculated.



Assay procedure

When diluting samples and reagents, they must be mixed completely and evenly. Before adding TMB into wells, equilibrate TMB Substrate for 30 minutes at 37°C. It is recommended to plot a standard curve for each test.

1. Set standard, **test samples (diluted at least 1/2 with Sample Dilution Buffer)**, control (blank) wells on the pre-coated plate respectively, and then, records their positions. It is recommended to measure each standard and sample in duplicate.

2. Prepare Standards: Aliquot 100ul of zero tube, 1sttube, 2ndtube, 3rdtube, 4thtube, 5thtube, 6thtube and Sample Dilution Buffer (blank) into the standard wells.

3. Add Samples: Add 100ul of properly diluted sample into test sample wells.

4. Incubate: Seal the plate with a cover and incubate at 37°C for 90 minutes.

5. Wash: Remove the cover and discard the plate content, and wash plate 2 times with Wash Buffer. Do NOT let the wells dry completely at any time.

6. Biotin-labeled Antibody: Add 100ul Biotin-labeled antibody working solution into above wells (standard, test sample and blank wells). Add the solution at the bottom of each well without touching the sidewall, cover the plate and incubate at 37°C for 60 minutes.

7. Wash: Remove the cover, and wash plate 3 times with Wash Buffer, and let the Wash Buffer stay in the wells for 1-2 minutes each time.

8. HRP-Streptavidin Conjugate (SABC): Add 100ul of SABC Working Solution into each well, cover the plate and incubate at 37°C for 30 minutes.

9. Wash: Remove the cover and wash plate 5 times with Wash Buffer, and let the wash buffer stay in the wells for 1-2 minutes each time.

10. TMB Substrate: Add 90ul TMB Substrate into each well, cover the plate and incubate at 37°C in dark within 10-20 minutes. (**Note:** The reaction time can be shortened or extended according to the actual color change, but not more than 30 minutes. You can terminate the reaction when apparent gradient appeared in standard wells.)

11. Stop: Add 50ul Stop Solution into each well. The color will turn yellow immediately. The adding order of Stop Solution should be as the same as the TMB Substrate Solution.

12. OD Measurement: Read the O.D. absorbance at 450nm in Microplate Reader immediately after adding the stop solution.

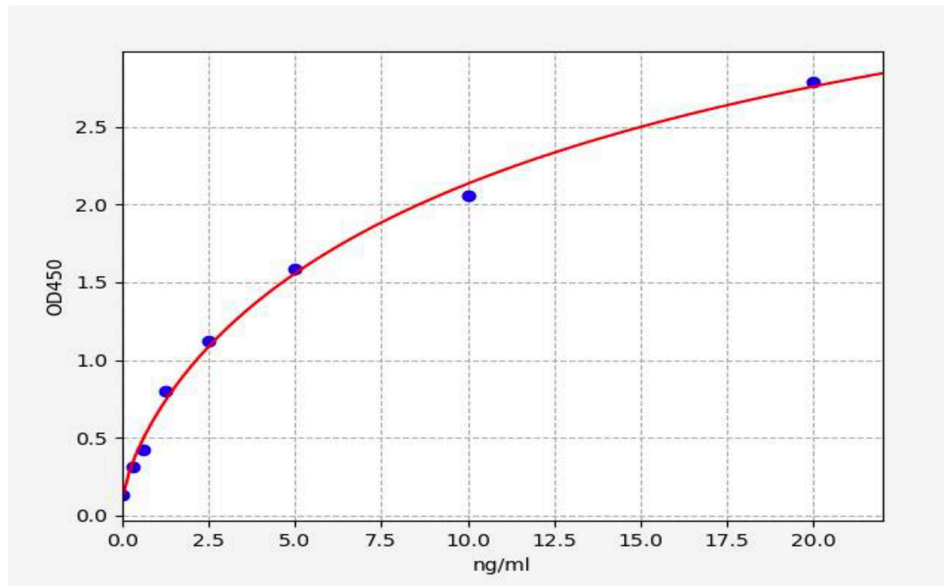


Figure 2-3: Total GSH calibration curve

Step1: Add 100ul standard or sample to each well and incubate for 90 minutes at 37°C.

Wash step: Aspirate and wash plates 2 times.

Step2: Add 100ul Biotin-labeled antibody working solution to each well and incubate for 60 minutes at 37°C.

Wash step: Aspirate and wash plates 3 times.

Step3: Add 100ul SABC Working Solution into each well and incubate for 30 minutes at 37°C.

Wash step: Aspirate and wash plates 5 times.

Step4: Add 90ul TMB Substrate Solution. Incubate 10-20 minutes at 37°C.

2.6.Statistical Analysis

The statistical analyses were performed utilizing the SPSS version 21 applications. The findings of Statistical tests were expressed as means ±SD. The studied parameter means were compared among the patient and control groups using a parametric independent t-test.. Because the Confidence interval (CI) of choice was 95%, the *P*-value of ≤0.05 was judged significant.

Results and Discussion

3.1 Serum levels of MDA

Mean serum MDA levels in the case and control groups were (3.1 ± 1.18 nmol/mL) and (1.07 ± 0.54 nmol/mL), respectively. The results showed significantly higher levels of serum MDA in the periodontitis group compared to the healthy control group. Mean and standard deviation of each data are presented in Table 1 and Figure(3-1)

Table (1): The Mean Serum Levels of MDA in Patients and Control

parameter	patient Mean±SD	Control Mean±SD	P-Value
MDA (nmol/mL)	3.1 ± 1.18	1.07 ± 0.54	P<0.001

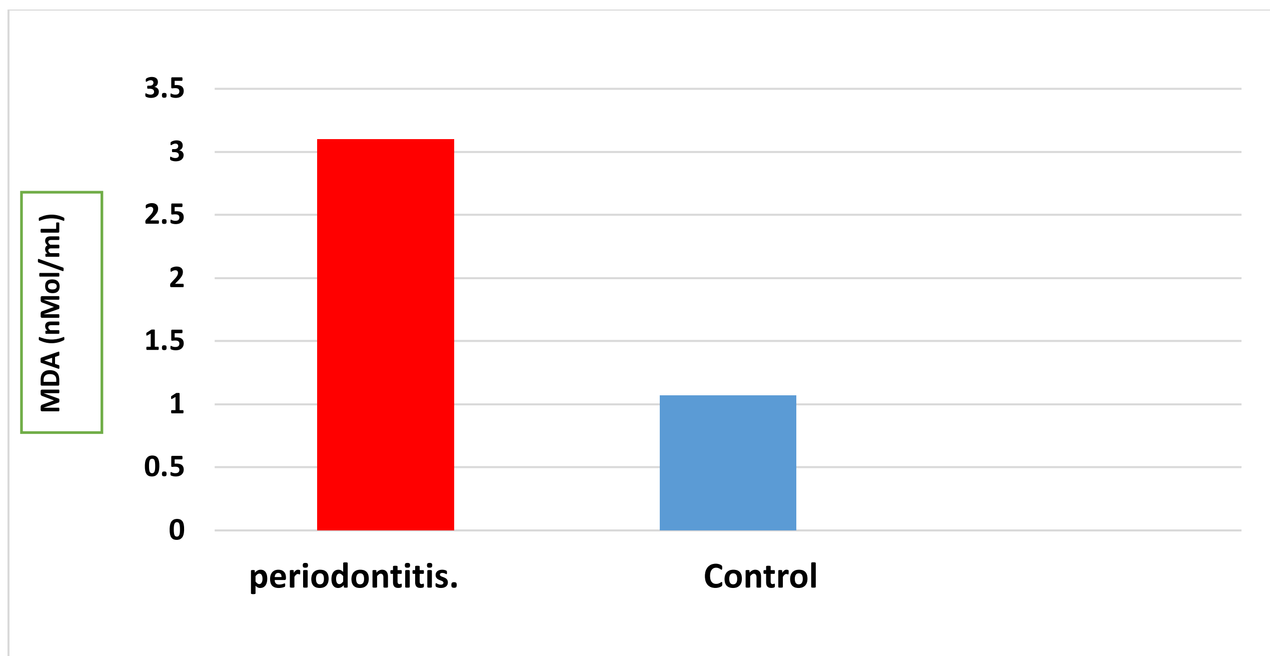


Figure (3-1): Comparison of serum Malondialdehyde level between control and periodontitis patient.

Malondialdehyde levels are increased in case of periodontitis patients whereas the level of Glutathione and superoxide dismutase are decreased when compared to healthy subjects. The reason for the increased levels of MDA is because, in case of periodontitis, there is an imbalance between the oxidants and antioxidants present in the oral cavity which ultimately leads to the production of ROS. Therefore lipid peroxidation occurs and there is increased MDA. For patients with periodontitis there is increased oxidative stress occurs which causes reduced antioxidant activity.

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.

Adult periodontitis is one of the most common chronic inflammatory diseases, in which microbial plaque causes periodontal ligament and bone destruction. Bacterial colonization, host immune response, and genetic predisposition are some of the main etiologic factors(Žilinskas et al., 2011). High oxidative stress and low antioxidant capacity might have important roles in the etiopathogenesis of periodontitis (Almerich-Silla et al., 2015); (Tamaki et al., 2015, Trivedi et al., 2015). We studied serum levels of MDA in chronic periodontitis. The results showed significantly higher levels of serum MDA in the periodontitis group compared to the healthy control group. Based on the results of this study, periodontitis can also induce systemic oxidative stresses and alter serum MDA levels and vice versa.

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(3-1). The antioxidant level in chronic periodontitis was 1.76±0.09 and that of normal patients was 1.15±0.18 (Baser et al., 2015). 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(Panjamurthy et al., 2005).

Consistent with the results of this study, in a study by Panjamurthy MDA level was significantly higher in the periodontitis group. Synthesis of MDA might be due to a decrease in AO in destroyed in periodontal tissues. Celec et al., too, showed high serum MDA levels in periodontitis. They concluded that local oxidative stress is a predisposing factor for MDA production in periodontitis(Celec et al., 2005). Trivedi et al. showed significant MDA elevation and reductions in antioxidant enzymes in periodontitis patients. They reported a direct correlation between MDA levels and an inverse correlation of antioxidant enzymes with periodontitis. Our results are consistent with studies demonstrating an increase in lipid peroxidation levels in serum, saliva, gingival crevicular fluid and gingiva in periodontitis(Trivedi et al., 2015).

3.2. Serum levels of GSH

Figure (3-2) &Table (2) shows the results of Glutathione levels in (serum a) samples of control and periodontitis patients. The results reflect a significant decrease ($P<0.0001$) in the serum level of GSH of periodontitis groups in comparison to that of the control.

Table (2): The Mean Serum Levels of Antioxidants in Patients and Control

parameter Antioxidants	**patient Mean±SD**	**Control Mean±SD**	**P-Value**
SOD(IU/mL)	92.41±22.59	188.38±26.55	P<0.0001
GSH (ng/mL)	4.00 ±1.33	11.47 ±3.03	P<0.0001

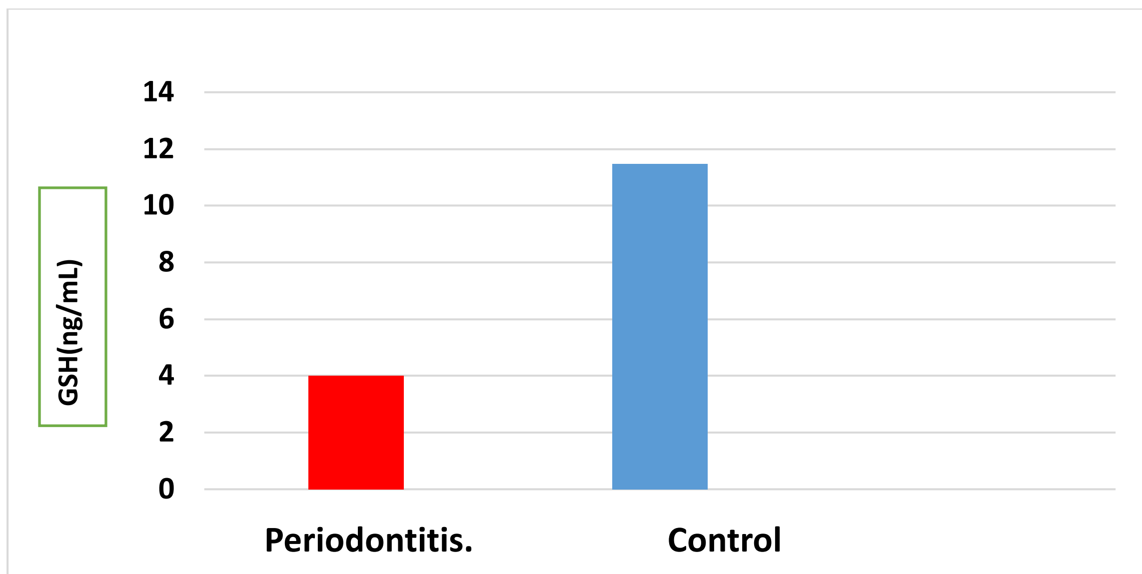


Figure (3-2): Comparison of serum Glutathione level between control and periodontitis patient.

Antioxidants like free radical scavenging enzymes are of importance in providing protection to normal cells and matrix components from oxidation and have a protective role to play in our body against oxidant stress. Previous studies(Kim et al., 2010)suggested that an imbalance in antioxidant-oxidant stress makes patients more susceptible to periodontal disease as the oxygen-derived species like hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH-$) cause cell injury, are involved in degenerative changes, and are often associated with increased peroxidative processes and linked to low antioxidant concentrations. This imbalance in the antioxidant-oxidant stress is also implicated in the pathophysiology of atherosclerotic disease process resulting in ischemic heart disease. The present study showed statistically significant decrease in the levels of the glutathione periodontitis patients(Chapple and Matthews, 2007). Comparatively higher levels of antioxidants were observed in the healthy group, which indicated that the oxidant related stress in the body is effectively balanced by the antioxidants. Lower levels of antioxidants in diseased states of chronic periodontitis and ischemic heart disease point towards the conclusion that the disease process is a result of a balance tipped in favor of the oxidants, with failure of antioxidants to balance the oxidant levels.In accordance with the studies done to evaluate the levels of glutathione there was varying results. In contrary to our study, A study done by, showed the levels of GSH was increased in the gingival crevicular fluid in patients with periodontitis. Not all patients demonstrated the increased

level of GSH in periodontal diseases . For eg. A study by Brock et al16 reported the levels of GSH was reduced when compared to healthy subjects. This indicates that there is hampered antioxidant capacity in patients with periodontitis. This study is in accordance with the our study. The levels of GSH might have a positive correlation between the progression of disease. (Lakshmi et al., 2009)

3.3. Serum levels of Total Superoxide Dismutase(SOD)

The mean value of sera total SOD activity in control and periodontitis patients groups are presented in Table (2) and Figure (3-3). These results show the presence of significant decrease (P<0.0001) in both periodontitis group in comparison to that of the control group.

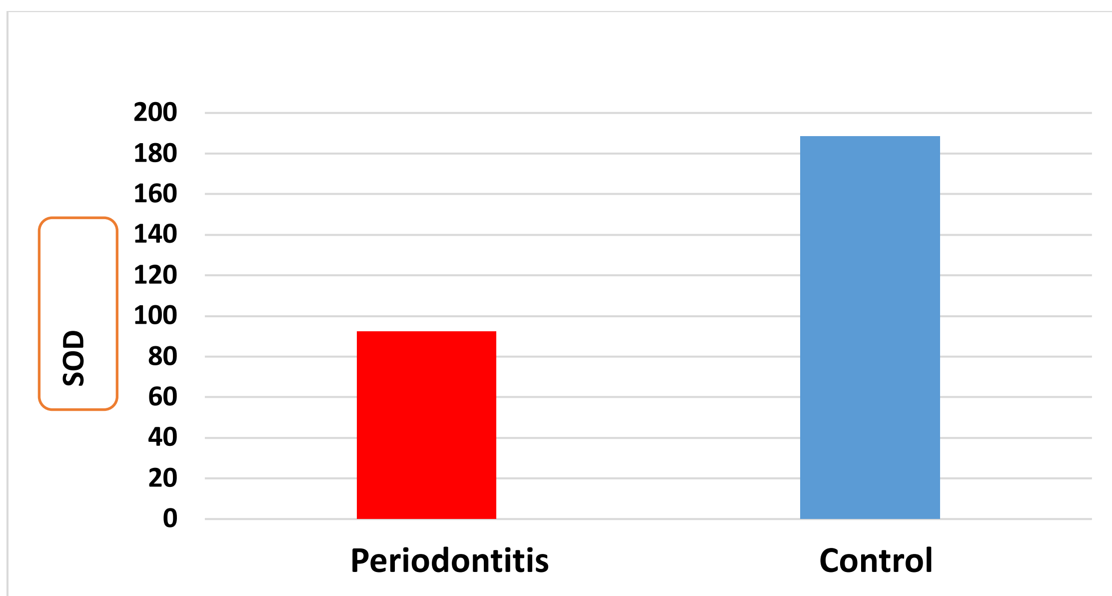


Figure (3-3): Comparison of serum Superoxide Dismutase level between control and periodontitis patient.

Antioxidant enzymes such as SOD provide the first line of cellular defense against ROS, protecting the cell against the production of other deleterious metabolites Antioxidants are known to treat various inflammatory conditions.

Potent free radicals attack on the oral mucosa leading to various alternations in a wide spectrum, from infection to lethal cancer (Punj et al., 2017). In the current work, a reduction in salivary SOD activity was observed, and this might be due to the depletion

of the antioxidant defense system. This could occur as a consequence of overwhelming free radicals in the
circulation of oral cavity cancer patients . Infiltration of immune system defense cell into lesion area of oral tumor has been reported, and killer activity emerged from these cells result in an increase in the concentration of free radicals (Karincaoglu et al., 2005). Dismutation of increased superoxide radicals, in particular, can be achieved by high SOD activity .
The increased activity of SOD result in overproduction of H_2O_2 which is the product of dismutation reaction, and it has been reported that H_2O_2 suppresses SOD activity and superoxide radicals $O^{2\cdot}$ †inhibit† catalase activity (one of the antioxidant enzymes that remove H_2O_2) . This may explain the observed reduction in serum SOD specific activity. 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 byproduct 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 (3-3). Similar reports are found in other studies too. 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(Punj et al., 2017).

4. Conclusion and Recommendation

4.1.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.

4.2.Recommendation

More comprehensive studies are needed for this research study and also subjects from different specificity of work and occupation should be considered. Further research is needed to confirm natural antioxidant therapies as definitive treatment for periodontitis.

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