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SALIVARY and SERUM OXIDATIVE STRESS and INFLAMMATION MARKERS in PERIODONTITIS PATIENTS

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STATEMENT

Hereby I declare that this thesis study is my work, I had no unethical behavior in any stage from the planning of the thesis until writing it, I have obtained all the information in this thesis within the academic and ethical rules, I have cited all the information and comments that are not obtained with this thesis study, and these sources are also included in the list of references, I hereby declare that I have no infringement of patents and copyrights during the study and writing of this thesis.

MOUSA MOHAMMAD ATEYAH

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II. CONTENTS

III. ABBREVIATIONS AND SYMBOLS

4-HNE: 4-Hydroxy-2-nonenal

ABTS: 2,2' -azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) radical cation

Antigen-presenting cells: APCs

AOs: Antioxidants

AP-1: Activating protein-1

BOP: Bleeding on probing

CAL: Clinical attachment level

CAT: Catalase

CRP: C-Reactive Protein

DCF: Dichlorofluorescein

GCF: Gingival crevicular fluid

GI: Gingival index

H_2O_2: Hydrogen peroxide

IFN-γ: Interferon gamma

IgE: Immunoglobulin E

LPS: Lipopolysaccharides

MDA: Malondialdehyde

mm: Millimeter

MMP: Matrix metalloproteinases

NF- kß : Nuclear factor-kß

OPG: Osteoprotegerin

OS: Oxidative stress

OSI: Oxidative stress index

PAMP: Pathogen -associated molecular patterns

PC: Protein carbonyls

PD: Probing depth

PI: Plaque index

PMN: Polymorphonuclear

RANKL: Nuclear factor kappa -ß ligand

ROS: Reactive oxygen species

SD: Standard deviation

SOD: Superoxide dismutase

SPSS: Statistical package for the social sciences

TAS: Total antioxidant status

TBARS: Thiobarbituric acid reacting substances is

TLR-4: Toll-like receptor

TNF-α: Tumor necrosis factor-α

TOS: Total oxidant status

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1. ÖZET

Tezin başlığı: Periodontitis hastalarında tükürük ve serum oksidatif stres ve enflamasyon markerlarının değerlendirilmesi.
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Programın Adı: Tezli Yüksek Lisans

Amaç: Bu kesitsel çalışma, evre III derece B/C periodontitisli hastaların tükürük ve serum örneklerinde total oksidan durumu (TOS), total antioksidan durumu (TAS) ve C-Reaktif protein (CRP) düzeylerini araştırmayı amaçlamaktadır.
Gereç ve Yöntem: Çalışmaya Marmara Üniversitesi Diş Hekimliği Fakültesi Periodontoloji Anabilim Dalı'na başvuran 80 kişi dahil edildi. Klinik ve radyografik değerlendirmelere dayanarak 40 bireye evre III, derece B veya C periodontitis teşhisi konuldu, 40 birey ise periodontal olarak sağlıklıydı. Çalışmada kayıt altına alınan klinik periodontal parametreler plak indeksi, gingival indeks, sondalama derinliği ve klinik ataşman seviyesidir. Serum ve tükürük TAS ve TOS, yüksek performanslı sıvı kromatografisi ile ölçülürken, serum CRP seviyesi CRP Türbidimetri Kiti ile belirlendi. Verilerin analizi SPSS programı kullanılarak yapıldı. İstatistiksel anlamlılık $p<0,05$ düzeyinde değerlendirildi.
Bulgular: Periodontitisli hastalarda CRP seviyesi kontrol grubuna göre anlamlı derecede yüksek bulundu ($p<0,05$). Periodontitis grubunda hem serum hem de tükürük TOS değerleri kontrol grubuna göre daha yüksek düzeylerde belirlendi ($p<0,05$). Ancak tükürük ve serum TAS değerlerinde gruplar arasında anlamlı bir fark bulunmadı ($p>0,05$). Oksidatif stres açısından evre III periodontitisli hastaların dereceleri arasında anlamlı bir fark görülmedi ($p>0.05$).
Sonuç: Bu çalışmanın sonuçları, oksidatif stresin periodontitiste periodontal sağlığa göre daha yüksek olduğunu göstermektedir.
Anahtar kelimeler: C-reaktif protein, oksidatif stres, periodontitis, reaktif oksijen türleri.

2. SUMMARY

Title of Thesis : Salivary and serum oxidative stress and inflammation markers in periodontitis patients.

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Program Name: Master of Science

Objective : The presented cross-sectional study aims to evaluate salivary and serum total oxidant status (TOS), total antioxidant status (TAS) and C-reactive protein (CRP) levels in patients with stage III grade B or C periodontitis and to compare them with periodontally healthy subjects.

Materials and Methods: The study included 80 participants admitted to the Department of Periodontology, Faculty of Dentistry, Marmara University. Based on clinical and radiographic examinations, 40 subjects were diagnosed with stage III, grade B, or C periodontitis, while 40 were periodontally healthy. Plaque index, gingival index, probing depth, and clinical attachment level were recorded as periodontal clinical parameters. TAS and TOS in both serum and saliva were determined by high-performance liquid chromatography. In addition, CRP in serum was determined using the CRP Turbidimetry Kit. Data were analyzed using the Statistical Package for Social Sciences (SPSS 25.0, IBM Inc., U.S.A.). Statistical significance was set at the level of $p<0,05$.

Results: A significantly elevated CRP concentration was observed in patients with periodontitis compared to the control group ($p<0,05$). Both salivary and serum TOS levels exhibited higher values in the periodontitis group ($p<0,05$). But, no difference was observed in both salivary and serum TAS levels between the groups ($p>0,05$). In addition, oxidative stress levels of stage III periodontitis were similar in grade B, and C patients ($p>0,05$).

Conclusion: The findings of the current study showed that subjects with periodontitis are more likely to be exposed to oxidative stress than periodontally healthy ones.

Keywords : C-reactive protein, oxidative stress, periodontitis, reactive oxygen species

3. INTRODUCTION and AIM

Periodontitis is an inflammatory disease that develops against biofilm-structured dental plaque and leads to loss of connective tissue attachment and alveolar bone of the tooth, eventually leading to tooth loss. This chronic, inflammatory disease affects at least 11% of the world's population (Richards, 2014).

Host response in periodontitis against microorganisms and their by-products causes gingival inflammation, connective tissue degradation, attachment and bone loss (Armitage, 1995). As it is well-known, the specific periodontopathogens colonized in subgingival biofilm are the primary etiological factors of periodontitis. However, the host response developed against these periodontopathogens has a crucial role in the progressive tissue destruction (Cekici et al., 2014, Meyle and Chapple, 2015). Therefore, to fully understand the etiopathogenesis of periodontal disease, the relationship between microorganisms and the host response should be explored.

In every organism, there is a homeostatic balance to repair vital tissue, cells, and molecular elements. Proteolytic enzymes of periodontopathogens and the inhibitory molecules in the periodontal tissues could be cited as examples of this homeostatic balance mechanism. Another mechanism of balance is between antioxidant defence systems, called antioxidants (AOs) and reactive oxygen species (ROS). ROS are free radicals that contain oxygen, and they also include non-radical oxygen compounds that participate in the creation of oxygen radicals, which contribute significantly to progressive tissue degradation (Lushchak, 2014). ROS-induced oxidation can be effectively delayed or even inhibited by the AOs existing in the cells (Sies, 1997).

Recently, the focus on ROS has grown in popularity and gained attention due to its significant role in many inflammatory diseases (Manish Mittal, 2014). Normally, ROS activity and AOs defense are in a dynamic balance. However, either by reducing AOs defense capability or increasing ROS production or activity, the equilibrium shifts in favor of ROS, which causes oxidative stress (OS) (Halliwell et al., 1992). A strong scientific proposal seems to link OS to the development of several inflammatory diseases, such as periodontitis (Chapple and Matthews, 2007, Pendyala et al., 2008) Considering the inflammatory degradation of extracellular matrix seen in periodontal

diseases, the impact of ROS on periodontal disease activity is not exactly clarified, and it is still an important issue worth studying.

Evaluation of functional activities of a particular oxidant or AO in a biological sample is not an easy approach due to cumulative effects and functional compensations between them. Moreover, serum concentrations of various AOs or oxidants can be determined via complex techniques in independent laboratories, but these measurements will be time-intense and costly. Therefore, total oxidation status (TOS) and total antioxidant status (TAS) are preferred to predict OS levels, described as the ratio between TOS and TAS, may be a more precise method of assessing oxidative stress. To elaborate more, TAS is a measurement frequently selected to evaluate the antioxidant level of biological samples and can estimate the antioxidant reaction against the free radicals produced in each disease, while total oxidant capacity is used to estimate the overall oxidation state of the body.

Numerous fundamental, experimental, and clinical research has found a strong association between OS and periodontitis (Ambati et al., 2017). A better knowledge of this relationship might provide us with various novel perspectives to clarify the pathogenesis of periodontitis. Studies have revealed that both OSI in fluids of the body, such as gingival crevicular fluid (GCF), saliva and serum, and TOS value are correlated positively with periodontal disease activity (Baltacıoğlu et al., 2014a, Erel, 2005). Aggressive periodontitis, which has more severe tissue destruction, was significantly correlated with greater TOS values and OSI than chronic periodontitis (CP). According to the case-control research conducted by Zhang et al., (Zhang et al., 2016) salivary and serum TAS of healthy subjects and CP patients were evaluated. The results of the aforementioned study indicated that the salivary and serum TAS levels of the CP group were lower than those of the healthy group.

OSI can provide recent information about the link between increased OS locally and /or systemically and increased periodontal destruction. Novel research reported in 2020 by Toczewska et al (Toczewska et al., 2020) aimed to assess total oxidative and antioxidative activity in both GCF and saliva for periodontitis patients and to compare with controls. The researchers found that in the unstimulated and stimulated saliva and GCF of the study group, higher OSI and TOS and lower TAS were detected.

The acute phase response characterized an initial complex reaction of the tissue toward inflammation. Numerous cytokines such as interleukins (IL), including (IL)-6 and IL-1α, trigger the liver cells to produce acute phase proteins in response to inflammation. As a result, blood levels of acute phase proteins such as CRP and amyloid rise. According to Gomes-Filho et al., (Gomes-Filho et al., 2011) there is a relationship between CRP levels and some diseases and conditions such as smoking, obesity, hyperlipidemia, diabetes, and periodontal disease. As a result, higher serum CRP levels can be predicted with periodontitis.

Periodontal tissue destruction, which is a consequence of host-microbial interactions, plays a crucial role in OS, which emerged as an outcome of excessive ROS activity or antioxidant deficiency, plays a crucial role in periodontal tissue destruction. Data regarding TAS and TOS are still conflicting and, according to our knowledge, until now, no published papers have discussed the TAS, TOS, and CRP amounts of the patients that were grouped according to a new classification of periodontal disease (Papapanou et al., 2018). Therefore, this cross-sectional study aimed to investigate serum and saliva TAS and TOS levels, and serum CRP levels of the stage III periodontitis patients with grade B or grade C.

4. GENERAL INFORMATION

Periodontal diseases are described as a set of chronic inflammatory disorders that affect the soft tissue surrounding the teeth, as well as the bone and periodontal ligament. Gingivitis is considered the initial amd mild form of periodontal disease and caused by the biofilm which grows on the surface of the teeth and around the gingiva. Inflammation is limited only to the gingivae and deep periodontal tissues are not affected. Periodontitis, on the other hand, is an inflammatory disease characterized by a complex biofilm interaction with the host's immune-inflammatory response, resulting in alterations in bone and connective tissue homeostasis. Periodontitis develops when gingivitis progresses to the bone and periodontal ligament. Thus, the destruction of periodontal tissues results in deep periodontal "pockets" that are the defining feature of the disease and can ultimately result in tooth loss (Kinane et al., 2017). The outcomes of various studies cited the potential effect of periodontal inflammation on systemic inflammatory conditions or diseases such as atherosclerosis and diabetes (Gotsman et al., 2007, Khader et al., 2006) .

4.1. Periodontal Disease Etiology

By the mid-1960s, it was known that the supra- and subgingival plaque, currently referred to as the microbial dental biofilm, were responsible for the development of periodontal disease. Dr. Harald Loe and colleagues (Löe et al., 1965) conducted a series of longitudinal clinical investigations to reveal if there is a relationship between plaque accumulation and the development of gingival inflammation. The first trial was carried out on a group of dental students who had better gingival health. Subjects were given dental prophylaxis and oral hygiene instructions before the experiment to achieve a plaque index (PI) and gingival index (GI) of zero, signifying that there was no visible plaque attached to their teeth and no clinically evident gingival inflammation. Subsequently, subjects were ordered not to brush their teeth for an extended period. The mean PI increased and the GI increased a few days later. They concluded that gingivitis is caused by the cumulative accumulation of dental plaque. Furthermore, when participants were instructed to resume brushing their teeth, the PI

was instantly reduced, and the GI decreased to regular levels several days later. Other research conducted on human participants revealed a shift in the microbiota composition from primarily aerobic Gram-positive microbes correlated with gingival health to anaerobic Gram-negative microorganisms (Löe et al., 1967, Theilade et al., 1966).

Understanding the nature of periodontal diseases with clinical and molecular evidence and classifying periodontal diseases based on them have always kept its place in the focus of periodontology. In 1973, Lindhe et al (Lindhe et al., 1973) supported Loe's findings with a study that allowed prolonged plaque accumulation in the beagle dog model. Eventually, the prolonged plaque accumulation led to GCF flow rate elevation, followed by an increase in gingival inflammation and an increased pocket depth with the development of gingival connective tissue breakdown. Finally, progression of soft tissue inflammation gingivitis is linked with an inflammation-mediated destruction of the teeth-supporting tissues named periodontitis.

The knowledge on the etiology of periodontal disease has been updated based on the findings that emphasized certain anaerobic microbes as causative. Numerous bacteria, particularly *Tannerella forsythia, Porphyromonas gingivalis, Treponema denticola, Prevotella intermedia, Aggregatibacter actinomycetemcomitans* and others, such as spirochetes, *Wolinella recta, Fusobacterium nucleatum,* have been correlated with severe types of periodontal disease (Haffajee and Socransky, 1994). Even now, the predominant view seems to be that the red complex *(P. gingivalis, T. forsythia, and T. denticola)* is the fundamental etiologic factor in the most common periodontal disease (Socransky et al., 1998) . Periodontitis is an inflammatory condition wherein severe forms are associated with aforesaid bacteria that have colonized the subgingival area despite the host's protective mechanisms. The host's response to microbial virulence factors such as endotoxin and lipopolysaccharide (LPS) is well-proven to be responsible for bone loss and connective tissue breakdown, which are essential features of periodontitis. However, the susceptibility of individuals appears to differ greatly depending on which risk factors are in effect (Genco, 1996). In addition to that, as Van Dyke (Van Dyke, 2014) summarized, the mutualistic relationship between these specific Gram-negative pathogens as well as the emergence and progression of periodontitis could produce the inflammation-modified environment. This ecological

change is leading to a shift of the microbial flora from a commensal Gram-positive one toward an anaerobic Gram-negative one.

4.2. Periodontal Pathogenesis and Histopathology

As a chronic multifactorial host-mediated inflammation, periodontitis leads to progressive damage to the structure of the teeth and loss of attachment. Plaque accumulation alone is sufficient for the onset of gingivitis. However, the initiation and progression of periodontitis depends on the existence of local, systemic, genetic, or environmental factors that make the host susceptible to the specific periodontopathogen microorganisms colonized in the plaque (Kinaneet al., 2017). These factors take part in an important function in ecological changes in the subgingival region, as well as in the challenge between colonized microorganisms in this region and the host-mediated antimicrobial mechanisms that develop against them. Inflammatory, several critical molecular pathways are activated, which are responsible for releasing host-derived proteinases. After that, apical translocation of the junctional epithelium and destruction of periodontal ligament fibers are observed, respectively. Consequently, attachment loss, deep periodontal pockets with gingival bleeding and radiological bone loss are observed as the common features of periodontitis (Papapanouet al., 2018, Tonetti et al., 2018) .

There are four distinct stages from healthy periodontium to gingivitis and from gingivitis to periodontitis, beginning with an initial lesion, through early and established lesions, to a histopathologicallyadvanced lesion (Kinane, 2001). Emergence of the first stage takes 2–4 days following plaque accumulation. Throughout this stage, exudative vasculitis is observed along the junctional epithelium, as well as polymorphonuclear (PMN) cell infiltration into the subgingival region through the junctional epithelium, loss of perivascular collagen, and fluid coexudation from the sulcus. The early phase occurs in 4 to 10 days. Concentrated T lymphocytes that penetrate fibroblasts are characteristic of this lesion. In 2 to 3 weeks, the development of the established lesion will take place. The characteristics of this lesion include increased B cells and loss of the gingival connective tissue matrix without any loss of hard tissue. Numerous PMN remains migrate over the junctional epithelium, gradually establishing the gingival pocket. Lastly, plasma cells continue

to dominate in advanced lesion, such as disrupted periodontium integrity, as well as in periodontal ligament and bone. This stage is distinguished by a change from junctional to sulcular epithelium, the creation of greater inflammatory infiltration formed of plasma cells and macrophages, a lack of collagen attachment to root surface (Page and Schroeder, 1976)(Bostanci and Belibasakis, 2018).

The healthy periodontium is capable of maintaining homeostasis through a variety of host immune system mechanisms (Cavalla et al., 2014). Innate, inflammatory, and adaptive immune responses are initiated once the equilibrium of the subgingival biofilm and infection control mechanism is lost. The persistent chronic inflammatory response to infection that affects the periodontium ultimately leads to irreversible loss of bone and, eventually, teeth (Kornman et al., 1997; Mira et al., 2017). (Bostanci and Belibasakis, 2018).

Inflammation is a defensive mechanism that manifests itself in a variety of ways, including swelling, redness, heat, pain, and impaired function (José & Flor, 2017).

Four points are important for the inflammation mechanism. The first is endogenous or exogenous reasons such as damage-associated molecular patterns, pathogen - associated molecular patterns (PAMP) and cell damage. Secondly, toll-like receptors (TLR) can recognize these molecular patterns. Pro-inflammatory mediators as the third one, include three main components: the complement system, cytokines, chemokines, and finally the target cells and tissues, on which the pro-inflammatory mediators take action (Muoz & Cordero, 2018).

The biofilm accumulation on the tooth surface can trigger the inflammatory host immune response. The biofilm adheres to the surface of the tooth, making the immune system incapable of effectively eradicating periodontopathogen microorganisms (Bostanci & Belibasakis, 2018). The first part of the periodontium to be exposed to bacteria is the junctional epithelium. Bacteria can pass through the junctional epithelium to gingival connective tissue, triggering the first response of inflammation (Cavalla et al., 2015, Noguchi et al., 2017). Bacterial PAMP, such as LPS, is then recognized by cells in the periodontium and is associated with TLR. TLR signaling pathways initiate, releasing various protein kinases that eventually result in activation of pro-inflammatory transcription factors such as activator protein 1 (AP-1) and

nuclear factor kappa -B (NF-κB). This activation encourages the release of mediators to mediate the inflammatory response(Han et al., 2015, Pöllänen et al., 2012).

Adaptive immunity has a substantial impact on the loss of bone in periodontitis via B and T lymphocytes. Neutrophils play a crucial role in periodontal lesion due to their production of cytokines and pro- or anti-inflammatory chemokines or their immunoregulatory features. These aforesaid features are directly linked to increased neutrophil amounts in the gingival sulcus, which have migrated across junctional epithelium (Chapple and Matthews, 2007). In addition, they can stimulate the migration of interleukin-17-producing CD4+ T helper (TH) 17 cells to inflammation sites as well as the maturation of B cells into antibody-secreting plasma cells. Additionally, stimulated neutrophils express a membrane-bound receptor activator of the nuclear factor kappa -ß ligand (RANKL), an essential cytokine that can induce hard tissue breakdown with its osteoclastogenic characteristics (Hajishengallis and Korostoff, 2017b). These well-described biological mechanisms indicate that neutrophils can influence the severity of periodontitis not only by starting the lesion, but also by contributing to its progression.

When the host response passes into the chronic state, the adaptive immune system responds by releasing various inflammatory and immunological mediators. These mediators are responsible for the signaling of the progression from gingivitis to periodontitis with their effects on bone homeostasis (Hajishengallis and Korostoff, 2017a). Research has indicated that these molecules are essential to activate the RANKL during periodontal inflammation (Dutzan et al., 2009). The cytokine RANKL stimulates osteoclastic activity by interacting with the RANK receptor that exists on the exterior of osteoclasts and osteoclast precursors (Nakashima et al., 2012). Osteoprotegerin (OPG) is a protein that can alter the biological effects of RANKL (Hofbauer et al., 2001). The upsurge in the RANKL/OPG ratio stimulates activation of osteoclast precursors, resulting in bone resorption (Menezes et al., 2008). Additionally, Th1 lymphocytes play a crucial role in the severity of periodontitis by increasing interferon gamma (IFN-γ) levels. Animal studies showed that subjects lacking IFN-γ-had lower amounts of infiltrated inflammatory chemokines and macrophages in periodontal tissue, resulting in less severe loss of alveolar bone (Garlet et al., 2008). Th1 cells also release cytokines such as IL-1β and TNFα. TNFα and IL-

1β actions include vasodilation, stimulation of endothelial cell activation to increase immune cell recruitment, stimulation of chemokine synthesis, neutrophil activation, and stimulation of MMP tissue activation. Additionally, Th2 lymphocytes are the primary source of IL-4, which stimulates immunoglobulin E (IgE) production in B cells and triggers macrophage activation in an IFN-γ- independent pathway (Gemmell et al., 2007). Finally, Th17 lymphocytes produce RANKL, which, in conjunction with inflammatory cytokines produced by Th1 lymphocytes, can alter bone metabolism and increase its resorption (Cardoso et al., 2009).

4.3. Oxidative Stress in Periodontal Disease

Periodontitis, as a common chronic dysbiotic disease, is characterized by host-mediated breakdown of inflammatory tissues. The alteration in host response to subgingival plaque biofilm yields periodontal tissue destruction. Over the last several years, the amount of research conducted to seek an answer about the role of OS in periodontitis etiology has been rising.

Cells are subjected to oxidants deriving from a huge range of exogenous and endogenous resources. Exogenous resources consist of smoking, trauma, heat, infection, and radiation. On the other hand, metabolic pathway by-products, electron leak from mitochondrial electron transport systems forming superoxide and functional production by host defense cells and connective tissue cells fall under the endogenous resources category. Oxidants, whether ROS or free radicals, act on the destruction of periodontal tissue. Excess ROS production plays a vital role in the initiation and development of various chronic inflammatory diseases, including periodontal disease (Figure 1) (Chapple and Matthews, 2007).

ROS molecules are required for numerous common biological functions. At the infected site, neutrophils produce superoxide ions. ROS includes reactive species that are not true radicals but are competent for radical development in both intracellular and extracellular environments. Free radicals are molecules capable of maintaining their self-stability that have one or more unpaired electrons. By their nature, they are extremely reactive species, efficient in withdrawing electrons and thus oxidizing numerous biomolecules crucial for tissue and cell activities. In cell culture medium, free radicals promote epithelial cell growth and fibroblast growth in minimal amounts,

but at elevated concentrations, they can cause tissue injury. The plaque contains different periodontopathogens that cause the release of a variety of interleukins and TNF from host cells. PMNs are drawn to the infection site by these pro-inflammatory cytokines. PMNs produce proteolytic enzymes as well as O_2 via an oxidative burst. Endogenous AOs (e.g., vitamin B, C, A, and E) together with enzymatic oxidants (e.g., superoxide dismutase, myeloperoxidase, and catalase) have been introduced as an antioxidant defense system of the human body. This antioxidant system reduces ROS into, to a lesser extent, reactive species, thus detoxifying it (Dahiya et al., 2013, Halliwell, 1991).

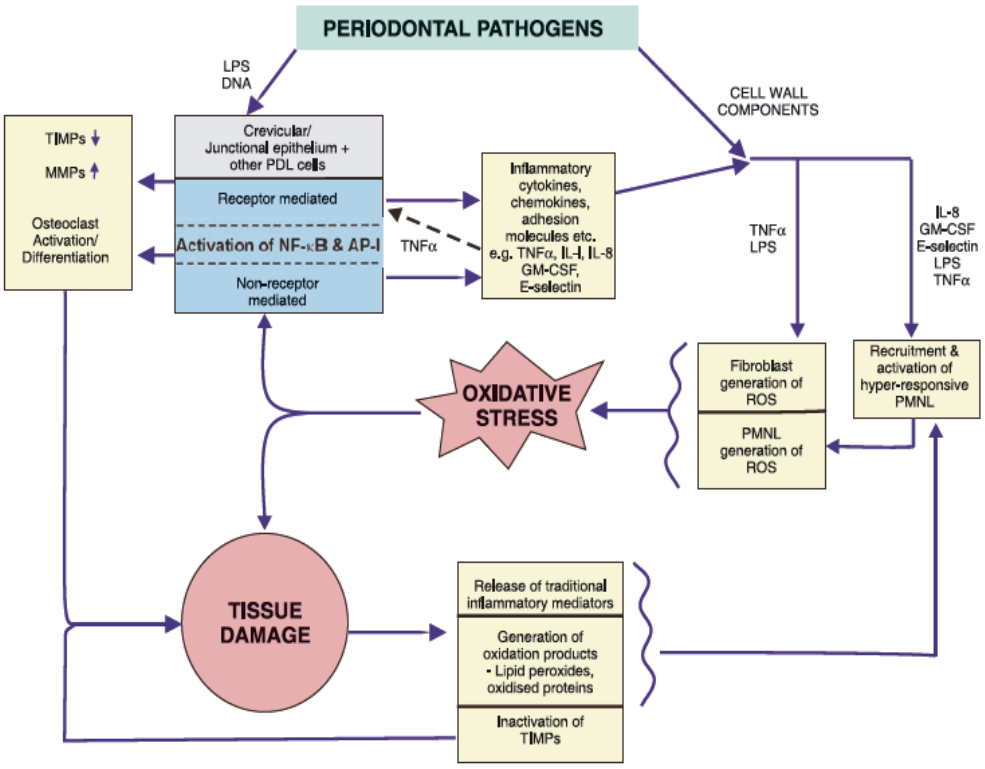


Figure 1: ROS has been shown to play a crucial role in tissue injury that happens during reacting against periodontal pathogens and the occurrence of chronic inflammation (Chapple and Matthews, 2007).

ROS in periodontal diseases is considered a double-edged sword. At minimal concentrations, ROS stimulates growth and differentiation in the culture of human periodontal ligament fibroblasts, while cytotoxic impacts on periodontal tissues may occur at higher levels and take part in pathogen killing (Luigi and Nikos, 2013).

ROS plays a physiological role in periodontal tissues. *P. gingivalis and Tannerella forsythia* are two keystone pathogens that can disrupt periodontal tissue equilibrium and cause inflammation (Wright et al., 2014).These pathogens are highly sensitive to variations in oxidative conditions. ROS can disrupt the cellular oxidative circumstances and aid in the elimination of principle pathogens (Lamont and Hajishengallis, 2015).

Nevertheless, ROS can act as second messengers, adjusting signal transduction, cellular homeostasis, and cell necrosis. Hydrogen peroxide (H_2O_2) may also surge the amounts of gelatinolytic matrix metalloproteinases (MMPs), which can promote human periodontal ligament fibroblast resettlement in a MMP-dependent manner (Cavallaet al., 2015).These results imply that ROS is involved in the differentiation and proliferation of human periodontal ligament fibroblasts. Numerous studies, however, indicate that H_2O_2 acts primarily as a suppressive mediator of cell proliferation and differentiation (Choi et al., 2009). A potential justification for these contradictory findings is that cellular responses to H_2O_2 can vary depending on the concentration and type of cell to which it is exposed (Burdon et al., 1996).

The immediate impact of ROS on connective tissue cellular structures has been widely investigated in the context of a broad diversity of inflammatory conditions and has been the topic of several publications (Canakçi et al., 2005, Chapple and Matthews, 2007). Additionally, the estimation of potential biomarkers for collagenous and noncollagenous connective tissue components in periodontal disease was recently reviewed (Embery et al., 2000, Giannobile et al., 2003). Furthermore, the impact of ROS on hard tissue resorption has not been extensively studied. However, certain ROS, such as superoxide and hydrogen peroxide, have been found to play a role in osteoclastic activation and consequently stimulate osteoclast formation (Bax et al., 1992, Garrett et al., 1990). At the ruffle-bone border, osteoclasts produce ROS, indicating an immediate participation in resorption (Key et al., 1994). The ability of

hydroxyl radicals and hydrogen peroxide to degrade alveolar bone proteoglycans in vitro supports this direct role in periodontitis bone resorption (Moseley et al., 1998).

ROSs are extremely active and have a very short life span. They can induce direct tissue damage, inflict irreversible DNA damage, cause lipid peroxidation, and release protein-damaging metabolites. These products are commonly used to assess ROS-induced tissue damage (Chapple and Matthews, 2007).

The by-products of lipid peroxidation are usually examined in periodontitis. Free radicals resulting from lipid peroxidation lead to variations in structural integrity and cell membrane function. Numerous lipid peroxidation metabolites, including malondialdehyde (MDA), 4-hydroxyl-2-115 nonenal (HNE), and isoprostane, were used to assess systemic and local oxidative harm associated with periodontitis.

MDA is a well-known lipid peroxidation by-product used to assess OS, and it is the most closely monitored lipid peroxidation outcome in periodontitis (Ahmadi-Motamayel et al., 2017). A procedure known as thiobarbituric acid reacting substances (TBARS) is utilized to determine MDA depending on the reaction with thiobarbituric acid. Periodontitis has been linked to higher levels of blood TBARS systemically and locally in GCF. (Panjamurthy et al., 2005).

Another major aldehyde product linked to lipid peroxidation is HNE (Petersen and Doorn, 2004). However, evidence of this molecule in periodontitis is rare.

Isoprostane, a by-product of arachidonic acid peroxidation, is frequently tested in urine serum, or plasma as an indicator of OS (Montuschi et al., 2004). A small number of studies have discussed isoprostane and its relation to periodontal disease (Wolfram et al., 2006).

ROS has the ability to cause polypeptide fragmentation or covalent crosslinking, which can alter functional protein activity (Shacter, 2000). Moreover, ROS could alter DNA by deconstructing purine and pyrimidine bases (Halliwell, 2000). In periodontitis, certain protein degradation caused by ROS was examined. Protein carbonyls (PC) are produced as end-products of protein oxidation by ROS and are measured using the carbonyl test. As an OS marker, PC has a significant benefit over lipid peroxidation products. Proteins that have been oxidized are more stable. PC develops early in life and circulates in the blood for an extended period of time. However, because protein-

bound aldehydes and glycated proteins are also detected, carbonyls are not specific indicators of ROS damage.

4.4. Antioxidant Defence Systems

Halliwell et al. (Halliwell et al., 1992) (1992) define AOs as substances that, when existing at smaller concentrations, are associated with those of oxidizable substrates and will hinder the oxidation of (Halliwellet al., 1992)those substrates AOs trap oxygen in the environment to inhibit the onset or progression of oxidation events (Young & Woodside, 2001). They play a vital function in maintaining the integrity of cells and tissues structure through allowing them to perform their regular tasks and guaranteeing the preservation of equilibrium among the antioxidant and oxidant mechanisms (Chapple and Matthews, 2007) (Figure 2).

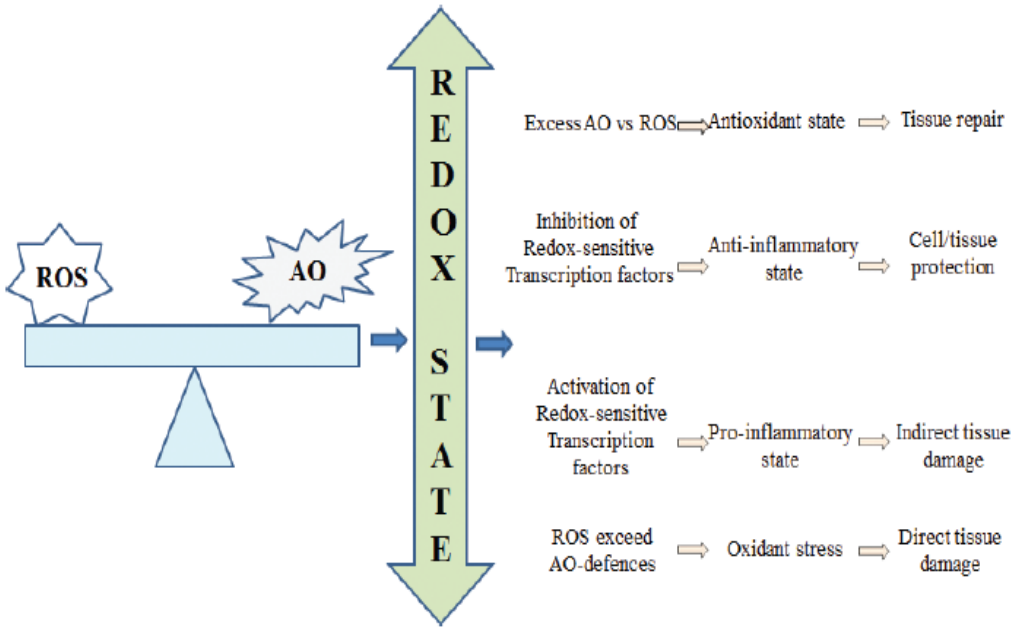


Figure 2. Biological effects of shifts on the equilibrium of activity among AO and ROS (Chapple and Matthews, 2007)

AOs can be classified based on the method of function, the place of action, the solubility, and their resources. The number one type consists of preventive AOs, including superoxide dismutase (SOD), catalase (CAT), and DNA repair enzymes, besides metal ion sequestrators like albumin. The other group includes AO scavenging

or chain-breaking AO such as carotenoid, uric acid, ascorbic acid, α-tocopherol, and polyphenols and reduced glutathione.

AOs can act against free radical damage through several mechanisms, including prevention of free radical formation, conversion of reactive metabolites to less reactive products, and facilitation of free radical damage repair. The antioxidant protection mechanism is extremely active and sensitive to alteration in the body's redox equilibrium. It prevents the production of free radicals that might occur because of OS. Jacoby and Davis demonstrated the presence of the ROS enzyme in the periodontal ligament using both biochemical and immunohistochemical techniques(Jacoby and Davis, 1991) . AOs show a solid protection function versus ROS; thus, some research attempted to analyze the effect of AOs in periodontitis treatments. Supplemental therapies with AOs, such as vitamin E, have been validated to improve clinical periodontal status (Singh et al., 2014, Sree and Sethupathy, 2014).

4.5. Measurement of oxidative stress in periodontitis

ROS are manufactured during metabolic and physiological activities, and they can cause destructive oxidative reactions in organisms that remove them via enzymatic and nonenzymatic antioxidative routes. Under specific instances, a rise in oxidants and a decrease in AOs are inevitable, and the oxidative-antioxidative balance shifts toward the oxidative status (Harma et al., 2005, Yanik et al., 2004).

Oxidant molecules are created endogenously in organisms and are also taken in from the surrounding environment. Endogenous ROS are produced by the electron transport chain and a variety of oxidase enzymes, including glycollate oxidase, xanthine oxidase, and monoamine oxidase. In a research facility, while serum or plasma levels of different both oxidant species and AOs can be detected independently, but measurements are time consuming, labor intensive, costly, and need specialized procedures (Tarpey et al., 2004). As a result, great caution is required to measure oxidant or AOs molecule levels seperately. Thanks to cumulative effects of both oxidative molecules and AOs, TOS and TAS of a sample could be computed. While TOS is defined the additive effects of different oxidative molecules, TAS, also known as total antioxidants capacity or activity, is described as additive antioxidant effects of AOs. (Ceylan et al., 2005, Harmaet al., 2005).

TAS was proposed as a low-cost method to evaluate the function of the complete antioxidant system (Chapple et al., 1997). Several assays test various AOs, some of which are lipophilic and others of which are hydrophilic; some assays evaluate preventive antioxidant systems, while others evaluate antioxidant scavenging. As a result, great caution must be exercised when interpreting the results of various tests that use distinct indicators of oxidative damage. (Yeum et al., 2004). Although significant relationships may be shown between disease state and individual antioxidants in biological systems, using techniques that use a distinct indicator of oxidative damage has significant disadvantages. For example, antioxidant systems act cooperatively and not in isolation. The total of the individual antioxidant activities does not signify their overall capacity to remove ROS. Interactions between hydrophilic and lipophilic antioxidants are not considered in these types of techniques. As a result of these factors, TOS tests have been created. (Aldini et al., 2001). In 2015, Erel developed a test to evaluate TOS in an acidic solution relying on the oxidation of ferrous ions to ferric ions in the presence of various oxidant species (Erel, 2005). Unlike prior approaches that concentrated on individual ROS or ROS products, the technique introduced may be utilized to detect the general oxidant status in a stable, cost-effective, and simple manner.

Another metric, OSI, was created to illustrate the amount of oxidative stress with the equilibrium of antioxidants. It is computed as TOS/TAS. These parameters have been extensively used to determine the entire OS correlated with periodontal disease.

Many of the connected investigations have revealed that periodontitis is associated with compromised local TAS. Furthermore, several experiments have found that periodontitis can affect circulating TAS. Plasma and saliva TAS levels were shown to be related to better periodontal parameters. Research on whether periodontal therapy might enhance local and/or circulating impaired TAS is conflicting (Guentsch et al., 2008). Hence, more precise research on the effects of periodontal treatment on local and systemic TAS is essential. Up to date, research found no correlation between TAS and bacterial load in periodontitis, indicating that TAS alterations may be associated with the immune host response rather than bacterial load (Zhanget al., 2016).

TAS can be linked with systemic variables like gender, smoking, pregnancy, and systemic disorders, which in turn can all relate to periodontitis. A few studies have

argued that males have raised serum TAS than females (Brock et al., 2004). Bakhtiari et al. (Bakhtiari et al., 2015) confirmed that salivary TAS among smokers is pointedly lower amid non-smokers. Another paper observed that neither gingivitis nor smoking affects salivary TAS (Aslan et al., 2014). In addition, investigations have indicated that periodontitis shows high values of TOS and OSI in saliva, GCF, and serum.

The aggressive subtype of periodontitis was associated with higher TOS and OSI values, which are connected with CP. Based on its significant correlation with clinical characteristics of periodontitis, a publication proposed OSI as the most recent biomarker for periodontitis. A 2017 research, on the other hand, found no difference in salivary TOS between the severe generalized periodontitis group and controls. TOS and OSI have been used to assess oxidative stress related to periodontal disease. Studies evaluating these parameters also validated amplified local and systemic OS with respect to periodontitis inflammation, but their sensitivity to being applied as biomarkers of OS correlated with periodontitis requires further confirmation.

4.6. Periodontal Disease and C-Reactive Protein

CRP is a plasma protein that is essential in the inflammatory response. It is an acute phase inflammatory marker that is sensitive and nonspecific and is generated in response to a variety of injuries (Black et al., 2004)(Black et al., 2004). Previously, CRP rates of > 10 mg/L were considered diagnostic for bacterial infection, while values <10 mg/L were ignored. This might be because CRP assessments previously were not as reliable and sensitive as they are now, making them less effective in detecting CRP levels of <10mg/L. As a result, laboratories can now detect CRP levels in serum as low as 0.15 mg/L using very sensitive CRP tests.

CRP is controlled by cytokines such as interleukin-6 (IL- 6) and tumor necrosis factor-α (TNF-α). Although CRP is an acute phase product formed by the hepatocytes in response to numerous inflammatory stimuli, it has been showed that its levels increase in periodontal disease. These cause systemic modifications involving hepatic production of a number of plasma proteins, stimulation of complement proteins, and numerous metabolic pathways (Ebersole and Cappelli, 2000, Kravitz and Shoenfeld, 2006). Moreover, it is considered a major biomarker for systemic inflammation (Panichi et al., 2000). Hege and Szalai (2007) demonstrated that CRP may bind

phosphoethanolamine and phosphocholine from damaged bacterial and host cell membranes, and also chromatin, small nuclear ribonucleoproteins, laminin, and fibronectin (Hage and Szalai, 2007) . Once CRP binds to ligands, it can initiate the complement cascade. Furthermore, because CRP receptors are found in monocytes, macrophages, and neutrophils, bound CRP can drive and exacerbate the ensuing local inflammatory response to trauma and infection.

The acute phase response is characterized by a dense sequence of physiological, nonspecific, systemic, and metabolic reactions that result in greater plasma protein synthesis and secretion. These modifications are referred to as acute because they occur within hours or days of the beginning of infection or injury, although certain acute phase alterations may reflect chronic disease. In acute inflammation, serum CRP quantities surpass 100 mg/L, and the level reduces in chronic inflammation.

Periodontitis occurs as a reaction to a bacterial infection caused by persistent inflammation initiated by plaque accumulation. However, the disease remains asymptomatic for a prolonged period of time and can only be identified by clinical examination (Slade et al., 2000). Gingival inflammation and periodontitis have been associated with increased amounts of acute phase proteins, indicating a locally stressed environment(Blacket al., 2004). However, not all studies have shown a relationship between destructive periodontitis and CRP. These findings might point to variations in the severity of destructive periodontal disease or disease development in different research groups.

According to recent research, individuals with severe periodontitis have higher blood CRP levels than the control group (Gomes-Filhoet al., 2011). Several studies have shown a connection between periodontitis and higher blood CRP levels because it is physiologically feasible that inflammatory mediators (IL-1, IL-6, and TNF- α) are generated during periodontal circumstances and have the ability to activate hepatocytes to produce CRP. Similarly, it is reasonable to expect greater blood CRP levels in the context of CP (Slade et al., 2003, Sladeet al., 2000). Reviewing the recent literature, in 2013, a study was conducted to compare salivary CRP concentrations of 90 patients, grouped as 30 healthy individuals, 30 gingivitis patients, and 30 subjects with CP. The results demonstrated a substantial change in salivary CRP concentrations between the periodontitis group and healthy controls. Researchers emphasized the

existence of a strong association between periodontal status and salivary CRP concentrations (Shojaee et al., 2013). In 2017, Ansari et al (Ansari Moghadam et al., 2017b) compared serum CRP levels of periodontitis patients with healthy controls. They concluded that periodontitis patients have higher systemic levels of CRP.

In the same year, Bolla et al (Bolla et al., 2017) compared serum CRP levels in healthy subjects, patients with CP, and aggressive periodontitis. Consistent with the studies mentioned above, they found higher levels of CRP in patients with CP and aggressive periodontitis than in healthy controls. Recently, in 2020, Anshul et al (Sawhney and Ralli, 2020) evaluated salivary and serum CRP levels in periodontitis and healthy controls. A total of 150 subjects were enrolled, 50 of whom were healthy, 50 of whom had CP, and 50 of whom had severe periodontitis. According to the findings, patients with aggressive periodontitis had the highest levels of salivary and serum CRP, followed by the CP group, and healthy individuals had the lowest.

In the light of all these information, the goal of this study is to determine the both serum and salivary TAS and TOS levels, and serum CRP levels of patients with stage III periodontitis and to compare them with healthy individuals.

5. MATERIALS and METHODS

The Clinical Research Ethics Committee of Marmara University, Faculty of Dentistry, approved the present study (No. 2020-55, Date:07.08.2020) (En 1). Each patient was fully informed about the data collection procedures and signed a consent form agreeing to participate in the study.

5.1. Study Groups

The present study included a total of 80 participants, including 40 patients with stage III grade B and C periodontitis and 40 periodontally healthy control individuals. Participants were selected among those admitted to the Department of Periodontology of the Faculty of Dentistry of Marmara University, Istanbul, Turkey.

All the individuals included in the study had the following characteristics: 1) were never-smokers; 2) had no history of systemic disease; 3) had received no periodontal treatment; 4) had not taken antibiotics, anti-inflammatory drugs, or any other drugs during the past 3 months; and 5) were not users of antioxidant vitamin supplement, any other over the counter remedies and alcohol.

Healthy group: The periodontally healthy subjects with the absence of any sign of clinical inflammation, not having periodontitis history; <10% of sites with bleeding on probing (BOP); PD ≤3 mm; absence of detectable attachment and/or bone loss; presence of 28 permanent teeth without extensive restorations or caries. In addition, all subjects in the healthy group did not show any local or systemic pathology.

Periodontitis group: Stage III, Grade B / C periodontitis patients were diagnosed on the base of the new classification criteria (Caton et al., 2018, Tonettiet al., 2018). Stage III periodontitis patients enrolled had ≥20 teeth, PD ≥6 mm, clinical attachment loss ≥5 mm, and bone loss reaching to the middle third of the root radiographically. Grade B was assessed by indirect consideration of radiographic bone loss throughout the most affected tooth in the dentition as a function of age (0.25-1.0), and Grade C was evaluated based on the radiographic bone loss in the most affected tooth in whole dentition as a function of age (>1.0).

5.2. Clinical Periodontal Examination

Plaque index (PI), gingival index (GI), probing depth (PD), clinical attachment level (CAL), and bleeding on probing (BOP) were recorded as clinical periodontal parameters. These data were documented in specially prepared data collection forms that included all teeth in the oral cavity except the third molars (En 3). During these procedures, the examination was completed with a 0.5 mm diameter and 15 mm periodontal probe (University of North Carolina, PCPUNC15, Hu-Friedy ins Co, USA) was used. Clinical periodontal measurements of the individuals included in this study were performed by a single dentist (MMA). Before starting the investigations, the researcher was calibrated internally to standardize the measurements within the research. Repeated measurements from 10 patients 3 days apart were taken and compared for calibration. The first measurement and the second measurement agreed with each other by 94.80%, and the Cohen's kappa value was found to be 0.93. This value shows that the agreement between the measurements is at its best level.

5.2.1. Plaque index

The Silness and Löe (Silness and Loe, 1964) PI was used to observe the oral hygiene levels of the patients. A score of 0-3 was given for each of the four surfaces of the teeth (buccal, midlingual, mesial and distal) with the subsequent scores and criteria.

Score 0: No plaque in the gingival area.

Score 1: No plaque is visible to the naked eye, but plaque is visible at the tip of the probe after moving it over the surface at the entrance to the gingival crevice.

Score 2: The gingival area is covered with a thin to moderately thick layer of plaque. The deposit is visible to the naked eye.

Score 3: Heavy accumulation of soft substances filling the gingival margin and tooth surface: the interdental area is accumulated with soft debris.

The scores of the four regions of the tooth were summed as the score of teeth. And the scores of the existed teeth were summed and divided into number of existed teeth to obtain the PI for the patient.

5.2.2. Gingival index

The gingival tissues were evaluated using GI (Loe and Silness, 1963) as a method of assessing the severity of gingival inflammation. Like PI, a score of 0-3 was given for each of the four surfaces of the teeth (buccal, midlingual, mesial and distal) with the subsequent scores and criteria.

Score 0: Normal gingivae.

Score 1: Mild inflammation: slight change in color, slight edema, no bleeding.

Score 2: Moderate inflammation: redness, edema and glazing, BOP

Score 3: Severe inflammation: marked redness and edema, ulceration; tendency towards spontaneous bleeding.

Bleeding was assessed by running a periodontal probe parallel to the long axis of each tooth along the soft tissue wall of the gingival crevice. The scores for the four areas of each tooth were summed as the score of teeth. The scores of existed teeth were summed and divided into number of existed teeth to determine the GI of the patient.

5.2.3. Bleeding on probing

BOP was performed at the following sites: mesio-buccal, mid-buccal, disto-buccal, mesio-lingual, mid-lingual, and distolingual. Probing was performed and 30 seconds later the site where bleeding occurred was marked with (+) or without bleeding was marked with (-). The percentage of BOP is calculated using the following formula:

BOP (%) = [(+) sites/ all sites] *100

5.2.4. Probing depth

PD, as the distance between the free gingival margin and the bottom of the pocket, was measured from a total of 6 sites in each tooth (mesio-buccal, mid-buccal, disto-buccal, mesio-lingual, mid-lingual, and disto-lingual). In order to obtain mean PD value of patient, The PD values of each site were summed and divided into total number of sites.

5.2.5. Clinical attachment level

CAL was obtained from the six sites (mesio-buccal, mid-buccal, disto-buccal, mesio-lingual, mid-lingual, and disto-lingual) for each tooth. CAL was recorded as the distance from the cementoenamel junction to the bottom of the pocket. In order to determinate the mean CAL value of a patient, the CAL values of each site were summed and divided into total number of sites.

5.3. Sample Collection and Biochemical Analysis

Unstimulated whole saliva samples were obtained in 5-minute periods while the patient was seated**.** Patients were instructed to allow saliva to pool on the bottom side of their mouth and drain into a collection tube.

Nine milliliters of blood samples from the antecubital vein were collected and placed in a centrifuge for 10 minutes at 5000 rpm to separate the anticoagulated blood into plasma and serum.

All samples were kept at – 80 ℃ conditions until the date of analysis. The all biochemical analysis was performed in the Biochemistry Laboratories of the Department of Biochemistry of the Faculty of Dentistry of Marmara University. Serum and saliva samples were investigated using a method introduced by Erel (Erel, 2005b; Erel, 2004). A more stable and colorful 2,2' -azinobis-(3-ethylbenzothiazoline-6-sulfonic acid**)** radical cation (ABTS) of a new generation was used. Antioxidants decolorize ABTS based on their concentrations and antioxidant capabilities; this color change is quantified as a change in absorbance at 660 nm. This procedure is carried out using an automated analyzer, and the assay is calibrated using Trolox. The test is based on the oxidation of ferrous ions to ferric ions in the presence of different oxidant species in an acidic solution, followed by xylenol orange detection of the ferric ions. The oxidation process of the assay was boosted, and protein precipitation was avoided. Furthermore, the autooxidation of ferrous ions in the reagent was inhibited during storage. The method was applied to an automated analyzer that was calibrated with hydrogen peroxide, and the analytical performance characteristics of the assay were determined.

CRP serum samples were also analyzed at the same laboratories. For this step, Samples were analyzed using by CRP Turbidimetric Kit (Bioanalytic Diagnostic Industry, LOT-923382N, REF: B80118, Expiray date: 2021) with Rayto brand Chemray 120 autoanalyzer device (China).

5.4. Statistical Analyses

Data were evaluated by employing SPSS for Windows, Release 25.0, IBM Inc., U.S.A.). Sample size estimation of this study was based on a previous study (Nguyen et al., 2020). When α=0,05 and β=0,10 with 95% power each group needed minimum 38 patients to detect differences within the two groups. For any possible dropout, 40

patients per group were included. Descriptive statistics were shown as number, percentage, mean and standard deviation. The distribution of data was evaluated with the Kolmogorov-Smirnov test to check normality. The Mann-Whitney U test was used for paired comparisons. The Spearman test was used for correlation analysis. Statistical significance was set at a $p<0,05$ level.

6. RESULTS

6.1. Demographic and Clinical Periodontal Parameters

The current study consisted of 40 patients with stage III, grade B, and C periodontitis (30 females and 10 males, aged 22 to 59 years) and 40 periodontally healthy controls (38 females and 2 males, aged 21 to 45 years). Table 1 presents the demographic characteristics of the study groups.

Table 1. Demographic characteristics of the study groups.

Parameters	**Control Mean±Sd N=40**	**Periodontitis Mean±Sd N=40**
Age (years)	29,30±5,65	38,51±8,49
Gender (F/M)	38/2	30/10

F/M: Female/Male; **N:** Counts; **Mean±Sd:** Mean±Standard deviation

As displayed in Table 2, all clinical periodontal parameters were elevated in the periodontitis groups than those in the control group. The mean values of PI, GI, and BOP were considerably higher in the periodontitis group ($p<0,05$). PD and CAL were 3,04±0,63 mm and 3,25±0,86 mm in periodontitis, and 1,88±0,39 mm and 1,91±0,40 mm in healthy subjects.

Table 2. Comparison of the clinical periodontal parameters between the groups.

Periodontal Parameters	**Control Mean±Sd N=40**	**Periodontitis Mean±Sd N=40**	**p***
PI	0,29±0,21	1,74±0,47	**<0,001**
GI	0,26±0,22	1,63±0,40	**<0,001**
BOP (%)	8,28±10,22	61,79±19,75	**<0,001**
PD (mm)	1,88±0,39	3,04±0,63	**<0,001**
CAL (mm)	1,91±0,40	3,25±0,86	**<0,001**

PI: Plaque index; **GI:** Gingival index; **BOP:** Bleeding on probing; **PD:** Probing depth, **CAL:** Clinical attachment level; **mm:** Milimeter; **%:** Percentage; **N:** Counts; **Mean±Sd:** Mean±Standard deviation, ***Mann -Whitney U test, $p<0,05$.

Table 3 shows the comparison of periodontal parameters between grade B and grade C periodontitis patients. There was no difference in PI, GI and BOP of periodontitis patients based on periodontitis grades (p>0,05), however PD and CAL mean values were statistically higher in Grade C patients than Grade B (p=0,02, p=0,009, respectively).

Table 3. Comparison of clinical periodontal parameters between stage III grade B and grade C periodontitis

Periodontal Parameters	**Grade B N=20 Mean±Sd**	**Grade C N=20 Mean±Sd**	**p***
PI	1,69±0,45	1,76±0,51	0,75
GI	1,59±0,42	1,67±0,37	0,39
BOP (%)	59,87±20,38	63,71±19,42	0,65
PD (mm)	2,82±0,65	3,18±0,57	**0,02**
CAL (mm)	2,99±0,68	3,52±0,95	**0,009**

PI: Plaque index; **GI:** Gingival index; **BOP:** Bleeding on probing; **PD:** Probing depth, **CAL:** Clinical attachment level; **mm:** Milimeter; **%:** Percentage; **N:** numbers; **Mean±Sd:** Mean±Standard deviation, *****Mann -Whitney U test, p<0,05.

The mean value of the CRP levels of healthy patients and periodontitis was 0,35±0,26 mg/L and 1,72±1,07 mg/L, respectively. A considerably higher CRP level was noted in patients with periodontitis in contrast to the healthy controls (p<0,001).

Table 5 illustrates the mean values of salivary and serum TAS, TOS and CRP levels in grade B and grade C periodontitis. Parameters investigated in saliva and serum were similar between grade B and grade C periodontitis (p>0,05).

Table 4. Comparison of salivary and serum biochemical parameters between groups.

Biochemical parameters	Control Mean±Sd N=40	Periodontitis Mean±Sd N=40	p*
TAS (serum)	3,03±2,36	2,50±2,18	**0,005**
TAS (saliva)	1,23±0,31	1,14±0,21	0,13
TOS (serum)	56,21±69,55	89,50±47,74	**0,04**
TOS (saliva)	30,5±19,36	45,86±33,52	**0,02**
CRP (mg/ l)	0,98±4,27	2,34±3,46	**0,001**

TAS: Total antioxidant status; **TOS:** Total oxidant status; **CRP:** C-reactive protein; **mg/l:** miligram/liter; **N:** Counts; **Mean±Sd:** Mean±Standard deviation; *Mann -Whitney U test, p<0,05.

Table 5. Comparison of salivary and serum biochemical parameters between grade B and grade C stage III periodontitis.

Biochemical parameters	Grade B N=20 Mean±Sd	Grade C N=20 Mean±Sd	p*
TAS (serum)	2,53±2,44	2,47±1,48	0,82
TAS (saliva)	1,11±0,18	1,18±0,22	0,53
TOS (serum)	95,58±61,84	83,42±26,28	0,41
TOS (saliva)	51,55±35,51	40,16±20,85	0,84
CRP (mg/ l)	2,12±3,55	2,55±3,45	0,26

TAS: Total antioxidant status; **TOS:** Total oxidant status; **CRP:** C-reactive protein; **mg/l:** miligram/liter; **N:** Counts; **Mean±Sd:** Mean±Standard deviation; *Mann -Whitney U test, p<0,05.

6.2. Correlations

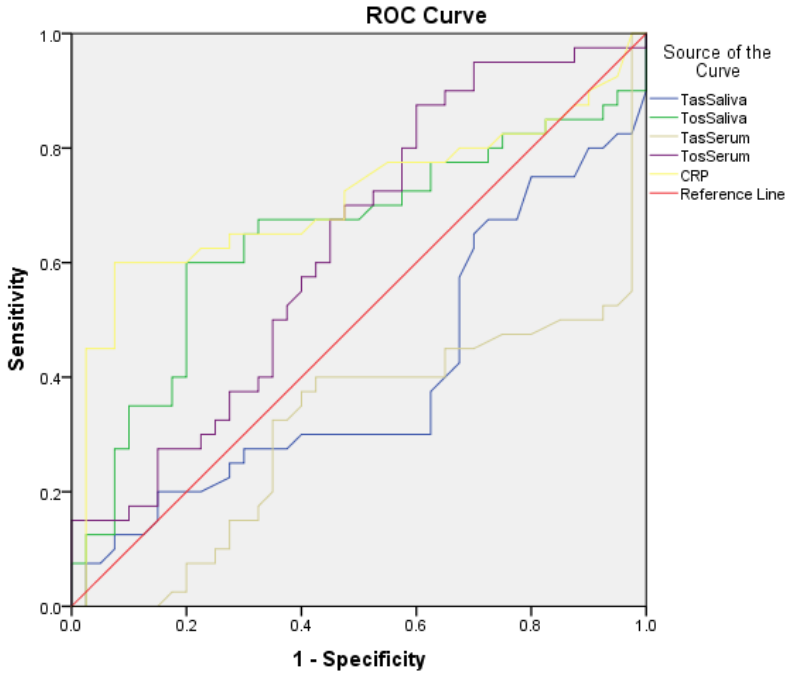
Associations between clinical variables and the serum and salivary TOS and TAS and serum CRP were explored together for all individuals (Table 6). Considerable associations were detected between the clinical periodontal parameters and serum TAS, TOS and CRP values (p <0,05). Similarly, a positive significant correlation was found between clinical periodontal parameters and salivary TOS, while no significant association was observed with salivary TAS.

Table 6. Spearman Rank Correlation Coefficients among the clinical periodontal parameters and serum and saliva TAS, TOS, and serum CRP levels in all participants

			PI	GI	PD	CAL	BOP	TAS Saliva	TOS Saliva	TAS Serum	TOS Serum	CRP
Spearman's rho	PI	Coefficient	1.000	,880**	,695**	,674**	,760**	-,124	,249*	-,225*	,197*	,249*
		Significance	.	,000	,000	,000	,000	,137	,013	,023	,040	,013
	GI	Coefficient	,880**	1.000	,698**	,696**	,815**	-,046	,284**	-,189*	,158	,278**
		Significance	,000	.	,000	,000	,000	,344	,005	,046	,080	,006
	PD	Coefficient	,695**	,698**	1,000	,953**	,813**	-,163	,270**	-,261**	,202*	,315**
		Significance	,000	,000	.	,000	,000	,074	,008	,010	,036	,002
	CAL	Coefficient	,674**	,696**	,953**	1,000	,792**	-,156	,242*	-,301**	,226*	,319**
		Significance	,000	,000	,000	.	,000	,084	,015	,003	,022	,002
	BOP	Coefficient	,760**	,815**	,813**	,792**	1,000	-,099	,307**	-,340**	,149	,284**
		Significance	,000	,000	,000	,000	.	,190	,003	,001	,093	,005
	TAS Saliva	Coefficient	-,124	-,046	-,163	-,156	-,099	1,000	-,044	,232*	-,240*	-,132
		Significance	,137	,344	,074	,084	,190	.	,350	,019	,016	,121
	TOS Saliva	Coefficient	,249*	,284**	,270**	,242*	,307**	-,044	1,000	-,234*	,124	-,079
		Significance	,013	,005	,008	,015	,003	,350	.	,019	,137	,243
	TAS Serum	Coefficient	-,225*	-,189*	-,261**	-,301**	-,340**	,232*	-,234*	1,000	-,309**	-,105
		Significance	,023	,046	,010	,003	,001	,019	,019	.	,003	,178
	TOS Serum	Coefficient	,197*	,158	,202*	,226*	,149	-,240*	,124	-.309**	1,000	-,008
		Significance	,040	,080	,036	,022	,093	,016	,137	.003	.	,473
	CRP	Coefficient	,249*	,278**	,315**	,319**	,284**	-,132	-,079	-.105	-.008	1,000
		Significance	,013	,006	,002	,002	,005	,121	,243	,178	,473	.

6.3. Reciever Operating Characteristics Curve

Figure 3 illustrates the Reciever Operating Characteristics Curve (ROC) curve of periodontal biomarkers. CRP was the biomarker with the highest AUC of 0,711 and a cutoff of 0,035ng/mL. Saliva and serum TOS and TAS saliva both had high AUC values of 0,629 and 0,645, respectively, although TAS saliva had a moderate AUC of 0,402. Only TOS serum, TOS saliva, and CRP showed a convex curve.



Periodontitis Stage III versus Periodontal health					
Biochemical parameters	**AUC**	**%95 CI**	**Cutt -off**	**SE-SP**	**p**
TAS (serum)	0.318	0.198-0.438	0.303	1-0.975	0.005
TAS (saliva)	0.402	0.275-0.529	1.05	0.675-.725	0.132
TOS (serum)	0.629	0.506-0.752	0.50	1-1	**0.04**
TOS (saliva)	0.645	0.520-0.771	9.915	0.900-0.975	**0.02**
CRP	0.711	0.590-.831	0.035	0.925-0.975	**0.001**

Figure 3. ROC curve for screening abilitiy of biochemical biomarkers for periodontitis stage III with AUCs, 95% CI, SE, SP and p values. p<0,05. **AUC:** Area under the curve; **95% CI:** Confidence of interval of 95%; **SE:** Sensisitivity; **SP:** Specifity; **TAS:** Total antioxidant status; **TOS:** Total oxidant status; **CRP:** C-reactive protein.

7. DISCUSSION and CONCLUSION

7.1. Discussion

Periodontitis is a prevalent chronic inflammatory disease in which plaque initiates the destruction of periodontal tissues. Periodontitis affects 10-15% of the adult population and if left untreated, leads to the loss of the supporting tissues of the teeth and the tooth ultimately (Chapple et al., 2007).

Leading causing factors for periodntitis includes bacterial colonization, host immune response, and genetic susceptibility (Pussinen et al., 2007, Žilinskas et al., 2011). The key etiological factor for periodontitis is predominantly gram-negative anaerobic or facultative bacteria within the subgingival biofilm (Kinaneet al., 2017). The cumulative plaque accumulation on the intraoral surfaces ends with the recruitment of leukocytes, mainly neutrophils, from the bloodstream to the site of infection. (Sczepanik et al., 2020). Neutrophils are considered as the initial line of defense against this disease and play a pivotal role in host defense. Neutrophils contain various selective methods for controlling bacterial invasion, including oxidative and nonoxidative killing mechanisms both intracellularly and extracellularly. The oxidative killing mechanism of neutrophils and other phagocytes involves the formation of ROS (Dahiyaet al., 2013). Recently, there has been increasing evidence about the role of ROS in emerging an oxidatively stressed environment that underlies the pathogenesis of a range of chronic inflammatory conditions, such as type 2 diabetes, atherosclerosis, and periodontitis (Sczepanik et al., 2020).

ROS may initiate damage to various cellular and extracellular tissues, causing protein damage, lipid peroxidation, and DNA damage leading to cell apoptosis. ROS induces periodontal tissue destruction through a variety of processes. These processes can be listed as extracellular matrix breakdown, protein oxidation, and excessive pro-inflammatory cytokine release mediated by NFκB activation (Chapple and Matthews, 2007). To prevent tissue damage caused by excessive ROS generation, cells require proper quantities of AOs (Neha, 2014). AOs are chemicals that, when present at low quantities in comparison to an oxidizable substrate, considerably delay or inhibit the oxidation reaction (Halliwell, 1990). Exogenous and endogenous sources of AOs include vitamins, minerals, enzymes, and hormones, as well as diet and herbal

supplements. Which may be in bar, gel, capsule, drops and tablet forms. Herbal therapy that contains antioxidant is used as complementary medicine to treat many medical and dental conditions. For the control of periodontal disorders, dental producers and distributors have recently incorporated AO supplements into toothpastes, mouth rinses/mouthwashes, fluoride gels and other dental products. Results from clinical studies, though incomplete, are positive. Furthermore, published research studies confirmed that AOs that work on skin cells also influence oral, gingival, and periodontal cells (San Miguel et al., 2010, San Miguel et al., 2011). AOs such as vitamin-E, vitamin-C, glutathione peroxidase, and superoxide dismutase protect tissue against free radical damage (Kurutas, 2016). OS occurs when hemostasis between AO defense system and ROS generation is disrupt. It was found that OS is linked to the pathogenesis of different diseases including periodontitis (Tóthová and Celec, 2017). Nearly, almost all inflammatory diseases lead to raised levels of OS. This in turn can trigger more damage to the tissues including the periodontal tissues and thus worsening periodontitis.

The negative effects of OS are known as oxidative damage, and they often arise after being exposed to a high quantity of ROS and/or a decrease in the antioxidant defense mechanism against ROS (Chapple and Matthews, 2007). When AOs are low, it appears that periodontal tissue's capacity to resist oxidative stress, retain normal tissue, and regulate bacterial damage is compromised. Low levels of most AOs are associated with a higher risk of periodontal disease and infection. (Pendyalaet al., 2008). Elevated OS and low antioxidant capacity may play a key title role in the etiology of periodontitis (Tamaki et al., 2015). OS biomarkers are therefore important molecules in terms of exact determination of disease status. A variety of approaches have been introduced to measure the OS. The ROS was estimated using direct techniques in the litreture. One of them is to estimate the cellular levels of ROS with a fluorogenic probes (Kim et al., 2018, Peshavariya et al., 2007). Because of their short lifespan and fast reactivity with redox status regulatory components, direct determination of ROS levels with high precision is challenging. As an alternate way to assessing OS in clinical samples, indirect assessment of ROS is utilized by analyzing the oxidative damage that these radicals cause to the lipids, proteins, and nucleic acids of the cells. Later, scientists established different new methods that can measure the OS of the body

through measuring the TAS and TOS. In 2005 (Erel, 2005), Erel introduced a beneficial method that was based on the oxidation of ferrous ion to ferric ion in the presence of various oxidant species in an acidic medium. Unlike previous techniques that focused on individual ROS or ROS products, this method can be utilized to determine the overall oxidant and AO state. Another indicator, OSI, was provided to illustrate the level of OS with the balance of AOs. It is calculated as TOS/TAS.

CRP is another critical inflammatory biomarker. Levels of CRP, a protein produced by hepatocytes and the chief protein of plasma, have a correlation with smoking, obesity, triglycerides, diabetes, and periodontal disease. The acute-phase response is the tissue's first and most complex reaction to inflammation. The majority of acute-phase proteins are produced predominantly by liver hepatocytes in response to pro-inflammatory cytokines such as IL-1α, IL-β, and IL-6. Serum concentrations of acute-phase proteins such as CRP increase with inflammation. In response to periodontal infection, the host produces cytokines and biological mediators such as ILs and prostaglandins. These cytokines are implicated in the destruction of periodontal tissues and generate a systemic acute-phase response. The serum levels of CRP rise very fast within 24 to 72 hours in conditions of inflammation or tissue damage and will decrease after the elimination of inflammation or infection. As reported by Gomes-Filho et al, an association was shown between CRP levels and some diseases / conditions listed as smoking, obesity, triglycerides, diabetes, and periodontal disease. As a result, elevated serum CRP levels might be predicted in the presence of periodontitis.

Since the significance of AO, OS, and inflammatory indicators in chronic diseases, particularly periodontal diseases. It is important and interesting to explore more in this field. In our study, we explored serum and salivary levels of TAS, TOS, and serum CRP in patients with stage III Grade B and Grade C periodontitis and healthy subjects. The study included a total of 80 participants, including 40 patients with stage III grade B and C periodontitis and 40 periodontally healthy control individuals.

The inclusion criteria were participants aged between 20 and 65 years old, nonsmokers, and systemically healthy subjects. Smoker subjects were excluded as smoking may enhance OS not only through the production of ROS in smoke but also through weakening of the AO defense systems (Isik et al., 2007). And this may affect the precision and goal of our study which focuses only on the evaluation of OS in

periodontitis. Furthermore, systemically diseased subjects were excluded since some of diseases may induce OS. For example, diabetes is a generator of OS. Hyperglycemia causes the formation of superoxide ions in endothelial cells at the mitochondrial level. Subjects with periodontitis were incorporated and classified them according to the most recent classification of periodontal diseases because, to our knowledge, no other study has evaluated OS in the contest for the new classification. The periodontitis patients were divided into two subgroups Grade B and Grade C. Grading aims to indicate the progression rate of periodontitis. In this study, different diagnostic measurements were used including panoramic radiographs and periodontal clinical parameters involving PI, GI, BOP, PD and CAL. Those diagnostic tools helped to reach an accurate diagnosis for periodontitis subjects. The all-clinical parameters in healthy subjects demonstrated lower values in comparison to the periodontitis group. This indicates the characteristic feature of our periodontitis subjects which is the destruction of the collagenous fibers of the periodontal ligament, resulting in loss of clinical attachment and bone support for the tooth. All periodontitis patients were diagnosed as stage III. The severity of staging a case depends on the greatest interdental clinical attachment loss and radiographic bone loss and the second component is grading which represents the rate of bone loss that has already occured and this was calculated using simple equation where the percentage of the bone loss was divided by the patient age. The percentage of bone loss in 20 patients was between 0.25 and 1 and those were stated as Grade B. In the other 20 patients the percentage of bone loss was greater than 1 and those were stated as Grade C.

TAS is widely recognized as one of the most convenient tests for evaluating how AOs affect the OS mechanism. We have shown an increase in the serum TAS among control subjects compared with the periodontitis group. Furthermore, there were no statistical variations in salivary TAS levels between the healthy and periodontitis groups. Although TAS data vary, it is generally accepted that with periodontal disease, serum and salivary TAS decline or remain unchanged (Baltacioğlu et al., 2006, Brocket al., 2004). Also, serum AO concentrations are inversely associated with the prevalence of inflammatory periodontitis (Chappleet al., 2007). The findings of the present study on TAS in periodontal disease and health are consistent with previous research (Akalin et al., 2009, Baltacioğluet al., 2006). Furthermore, the literature implies that the serum

TAS is lower in periodontitis than in periodontal health (D'Aiuto et al., 2010, Konopka et al., 2007).This can be explained by the fact that the immune system's inactivity against the periodontal pathogens leads to an increase in ROS products from neutrophilic granulocytes. To counteract the tissue destruction of ROS, a large amount of antioxidant components must be used to neutralize the ROS, which is probably the reason why TAS levels decrease or remain unchanged in periodontitis patients. In contrast, serum TOS rates were more in the periodontitis subjects in relation to the control group. Additionally, the periodontitis group presented an increased salivary TOS value. This result was prallel with previous studies in which an increase in TOS was observed in periodontitis (Akalin et al., 2007, Baltacıoğlu et al., 2014b, Wei et al., 2010). The increase in TOS levels in saliva reflects the increase in ROS radicals. A study using TOS as an indicator to track the effectiveness of nonsurgical periodontal treatment of periodontitis showed that TOS had a reduction after treatment. (Weiet al., 2010). This outcome appears to indicate that OS is elevated in periodontitis rather than in the control healthy group. Controversy, Zhang et al (Zhanget al., 2016) did not show any association in salivary TOS concentrations between patients with periodontitis and the control group.

A drastically higher CRP amount was identified among subjects with periodontitis in relation to the control group, showing that an inflammatory protein may help to gain a fuller knowledge of the health of periodontal tissues during and after periodontal treatment. CRP levels are associated with the inflammatory conditions such as cardiovascular disease and rheumatoid arthritis. CRP levels were observed to be increased among individuals with periodontitis compared to healthy controls. Salivary CRP concentrations of patients which were grouped as 30 healthy subjects, 30 gingivitis, and 30 chronic periodontitis patients, displayed a substantial variance in salivary CRP concentrations between the periodontitis patients and controls, and a strong association between periodontal status and salivary CRP concentrations (Shojaee et al., 2013). In 2017, Ansari et al (Ansari Moghadam et al., 2017a) concluded that periodontitis patients have higher systemic concentrations of CRP. Also in the same year, Bolla et al (Bollaet al., 2017) found higher serum levels of CRP in both chronic and aggressive periodontitis patients than in healthy controls. Sawhney et al (Sawhney and Ralli, 2020) published a study in 2020 that investigated both salivary

and serum CRP levels in 50 participants with aggressive periodontitis, 50 people with chronic periodontitis, and 50 healthy people. According to the data, aggressive periodontitis patients had the highest levels of salivary and serum CRP, followed by chronic periodontitis patients, while healthy people had the lowest levels.

Although the parameters were examined associated with the clinical periodontal status, TAS, TOS, and CRP did not distinguish between individual grades of periodontitis. The TOS and TAS observations are consistent with the findings of Joanna et al (Toczewskaet al., 2020). Regarding the ROC curve, saliva and serum TOS and TAS saliva both had high AUC although TAS saliva had a moderate AUC. Only TOS serum, TOS saliva, and CRP showed a convex curve. The concave curve represents a biomarker with little diagnostic capacity. The convexity of the ROC curve grows as the biomarker diagnostic power increases (Inpyo et al., 2020). To the best of the author's knowledge, this is the first study to compare serum TAS and TOS, salivary TAS and TOS, and serum CRP levels in patients with Stage III and Grade B and C periodontitis with healthy participants. Our results confirmed that OS and CRP are higher in periodontitis compared to healthy subjects.

Not collecting GCF samples and taking only saliva and serum samples can be considered as a limitation of this study. Although saliva and serum are significant diagnostic method, some problems were introduced. Sometimes, patients have problems with saliva collection, some require a long time until enough sample volume is reached, which might bias the measured concentrations. The connective tissues of the periodontium are highly vascular to faciliate migration of the molecular and cellular components of serum into the periodontium; thus, the gingival crevice is bathed with GCF. GCF is a serum exudate that includes key molecules such as complement components, antibodies, neutrophils and plasma cells components of the immune response required to prevent tissue invasion by the subgingival colonized bacteria. Furthermore, not conducted of the comparison of periodontitis in terms of different stages can be listed another limitation. Since comparing different stages can get a wider overview on the relation between OS and the severity of the disease. Salivary and serum TAS, TOS, and CRP were suggested to be related with more severe and aggressive forms of periodontal disease.

Recently, there has been rising demand in disease prevention using certain AOs as dietary supplements (Panjamurthy et al., 2005; Canakci et al., 2007); therefore, more investigations are recommended on larger populations with different periodontal status to recognise the mechanisms involved and whether they are the effect or cause of the disease.

7.2. Conclusions

According to the results of the current study, OS levels were found to be higher in periodontitis patients than in periodontally healthy individuals. Based on these conclusions and the relationships between TOS and periodontal health, TOS could potentially be a valuable and practical indicator of oxidative destruction in periodontitis. Although OS is a well-accepted fact that it plays a significant role in the pathological process of periodontal disease, there have been only a limited number of studies that focus on how it affects periodontitis. Consequently, it is possible that further research results concerning the OS grade progression in periodontitis would provide alternative perspectives as to why periodontal infections pursue chronic and/or aggressive courses. In this regard, to the best of our knowledge, this is the first study to compare TAS, TOS, and CRP across different periodontitis grades. The results of the current study show that OS levels in periodontitis patients were higher than those in periodontally healthy individuals, showing no significant differences among periodontitis grades.

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Hasta takip formu

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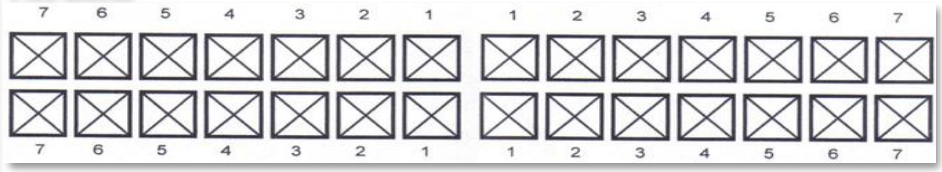

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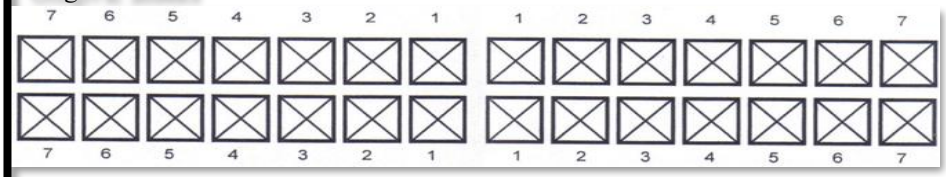

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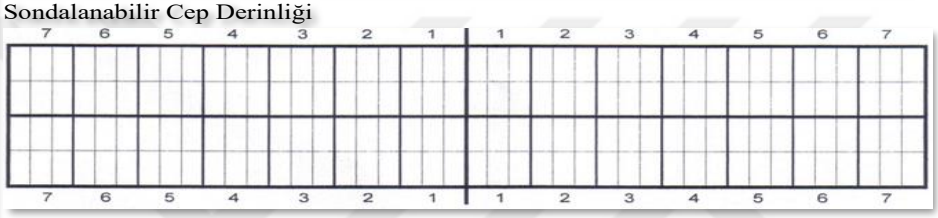

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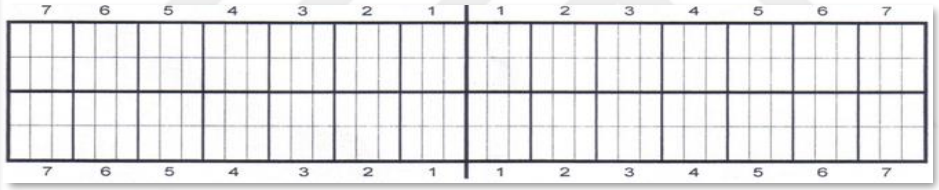

Sondalamada Kanama