REPUBLIC OF TURKEY
HARRAN UNIVERSITY
INSTITUTE OF HEALTH SCIENCES
DEPARTMENT OF MEDICAL BIOCHEMISTRY

EXAMINATION OF 8-OHDG, CRP, IL-21 AND OXIDATIVE STRESS LEVELS IN THE SERUM OF YOUNG CHILDREN WITH EARLY CHILDHOOD DENTAL CARIES

MASTER THESIS

Enes ELMUHAMMED

SUPERVISOR

Assoc. Prof. Dr. Nihayet BAYRAKTAR

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This work was not supported by any institution

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ACKNOWLEDGEMENTS

I want to thank Assoc.Prof.Dr. Nihayet BAYRAKTAR' for her efforts throughout the research and for her endless support. Her guidances lighten my path. I would also like to thank Prof.Dr. Mehmet BAYRAKTAR' for his valuable information that helped enrich my research. I would also like to thank Assoc. Prof. Dr. Mehmet Sinan DOĞAN for his contribution to the completion of the research. In Addition, I would like to thank my family for their support all the time, and also my friends who helped me.

ENES ELMUHAMMED

2022

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SYMBOLS and ABBREVIATIONS

CMV : Cytomegalovirus

CRP : C-Reactive Protein

DNA : Deoxyribonucleic Acid

ECC : Early Childhood Caries

ELISA: Enzyme-Linked ImmunoSorbent Assay

IL-21 : Interleukin-21

MDA : Malondialdehyde

8-OHdG: 8-Hydroxy-2' -Deoxyguanosine

OSI : Oxidative Stress Index

PMN : Polymorphonuclear Leukocytes

ROS : Reactive Oxygen Species

RNS : Reactive Nitrogen Species

SOD : Superoxide Dismutase

TAS : Total Antioxidant Status

TAC : Total Antioxidant capacity

TFH : Follicular Helper T Cells

TOS : Total Oxidant Status

TNF-α : Tumor Necrosis Factor Alpha

WHO : World Health Organization

ÖZET

ERKEN ÇOCUKLUK ÇAĞİ DİŞ ÇÜRÜKLERİ OLAN KÜÇÜK ÇOCUKLARIN SERUMUNDA 8-OHDG, CRP, IL-21 ve OKSİDATİF STRES SEVİYELERİN İNCELEMESİ

Enes ELMUHAMMED

Tıbbı Biyokimya Anabilim Dalı, Yüksek Lisans Tezi

Diş çürüğü, diş plağındaki bakterilerin ürettiği asitlerin neden olduğu diş kaybı veya dişlerde lokal hasar ile karakterize olan en yaygın ağız hastalıklarından biridir. Erken çocukluk çağı çürükleri (ECC), 71 aylık veya daha küçük çocuklarda süt dişlerinin diş çürümesini tanımlamak için kullanılan terimdir. Erken çocukluk çağı çürükleri (ECC) hala çocukluk çağında en sık görülen hastalıklardan biridir. Uyumu artıracak ve genel terapötik sonucu iyileştirecek diş çürüğünün ilerlemesini izlemek için invazif olmayan yöntemlere ihtiyaç vardır. Bu durum diş çürükleri ile ilişkisini kanıtlayan biyokimyasal indikatörlerin kullanılmasını uygun kılmaktadır. Çocuklarda diş çürüklerinin erken teşhisi için daha etkili ve daha az invaziv yöntemlerin araştırılması gerekmektedir. Bu daha az tedavi prosedürüne izin verir. Bu nedenle, çalışmamız erken çocukluk çağı çürüğü olan çocukların serumunda TAS, TOS, OSI, 8-OHdG, IL-21 ve CRP düzeylerini ölçmeyi amaçlamaktadır. Çalışmamızda erken çocukluk çağı çürüğü olan 45 çocuğun (n=45) tedaviye başlamadan önce serumlarını inceledik. Hastalar, tedavi için Harran Üniversitesi Diş Hekimliği Fakültesi Çocuk Diş Hekimliği Anabilim Dalı'na geldiler. Hastalıkları teşhis edildi ve genel anestezi altında tedavi edildi. Sağlıklı kontrol grubu olarak diş çürüğü, periodontal enfeksiyon veya sistemik hastalığı olmayan 45 çocuğu seçtik (n=45). Serum 8-OHdG ve IL-21 seviyeleri ELISA yöntemi kullanılarak ölçüldü. TOS, TAS ve CRP seviyeleri spektrofotometrik olarak belirlendi. Daha sonra oksidatif stres indeksi hesaplandı. Çalışma sonucunda erken çocukluk çağı çürüğü olan hastaların serumunda TOS, CRP, 8-OHdG, IL-21 ve OSI düzeyleri sağlıklı kontrol grubuna göre yüksek iken Erken çocukluk çağı çürüğü olan çocukların serumunda TAS düzeyi sağlıklı kontrol grubuna göre düşük bulundu

($p<0.001$). Erken çocukluk çağı çürüğü olan hastaların, serum 8-OHdG düzeyleri ile TAS arasında orta düzeyde pozitif korelasyonlar gözlendi ($P<0.05$). Ek olarak, TOS ve OSI serum seviyeleri arasında anlamlı ve güçlü pozitif korelasyonlar vardı ($P<0.01$). Sonuçlarımız, diş çürüğünün mekanizmasını daha iyi anlamaya ve diş çürüğünün ağız boşluğu dışındaki yolunu tahmin etmeye yardımcı olabilir. Biyobelirteçlerin dental prosedürlerde yaygın kullanımı, çok çeşitli bulgular ve analizler üretebilir. Erken çocukluk çağı çürükleri ile serum biyokimyasal göstergeleri arasındaki ilişkiyi daha iyi açıklamak için daha fazla araştırma yapılmasını öneririz.

Anahtar Kelimeler: ECC, 8-OHdG, IL-21, Oksidatif Stres, CRP

ABSTRACT

EXAMINATION OF 8-OHDG, CRP, IL-21 AND OXIDATIVE STRESS LEVELS IN THE SERUM OF YOUNG CHILDREN WITH EARLY CHILDHOOD DENTAL CARIES

Enes ELMUHAMMED

Department of Medical Biochemstery, Master Thesis

Dental caries is one of the most prevalent oral diseases and is characterized by tooth loss or localized damage to the teeth caused by acids produced by bacteria in dental plaque. Early childhood caries (ECC) is the term used to describe tooth decay of the primary teeth in children who are 71 months of age or younger. Early childhood caries (ECC) is still one of the most common diseases in childhood. Non-invasive methods are needed to monitor the progression of dental caries, which will improve compliance and the overall therapeutic outcome. This makes it appropriate to use biochemical indicators that prove their relationship with dental caries. It is necessary to search for more effective and less invasive methods for early detection of dental caries in children, which allows for fewer treatment procedures. Therefore, our study aims to measure the levels of TAS, TOS, OSI, 8-OHdG, IL-21, and CRP in the serum of children with early childhood caries. In our study, we examined the serum of 45 children with early childhood caries before starting treatment (n = 45). They came to Harran University, Faculty of Dentistry, Department of Pediatric Dentistry, for treatment. Their disease was diagnosed and treated under general anesthesia. We chose for the healthy control group 45 children who did not suffer from dental caries, periodontal infections, or systemic diseases (n = 45). The serum 8-OHdG and IL-21 levels were measured using the ELISA method.TOS, TAS, and CRP levels were determined spectrophotometrically. Then the oxidative stress index was calculated. As a result of the study, the levels of TOS, CRP, 8-OHdG, IL-21, and OSI increased in the serum of patients with early childhood caries compared to patients in the healthy control group ($p<0.001$). While the level of TAS decreased in the serum of children with early

childhood caries compared to children in the healthy control group ($p<0.001$). In the early childhood caries patients, medium positive correlations were observed between serum levels of 8-OHdG and TAS ($P<0.05$). Additionally, there were strong significant positive correlations between serum levels of TOS and OSI ($P<0.01$). Our results may help us better understand the mechanism of dental caries and predict its traces outside the oral cavity. The widespread use of biomarkers in dental procedures can produce a wide range of findings and analyses. We recommend further research to better explain the association between early childhood caries and serum biochemical indicators.

Keywords: ECC, 8-OHdG, IL-21, Oxidative Stress, CRP.

1. INTRODUCTION

The general health of the human body is significantly influenced by dental health. Proper mastication performed by the teeth enables proper digestion of the food which in turns helps in the proper development of the human body. Besides, the aesthetics function the teeth provides to the individual.

Since childhood is an important stage in the formation of the body, the defect in the function of the teeth leads to many consequences. One of the most important diseases affecting teeth in childhood is Early Childhood Caries (ECC) (4).

It is regarded as one of the most widespread illnesses affecting children worldwide. In Germany, the prevalence of ECC is about 10% in children aged three years, while this percentage reaches 50% in children aged six and seven years. In the United States, the prevalence rate is 40% among children aged 2-11 years (4).

In 2015, the number of infected children reached 560 million children of all ages. Thus, early childhood caries ranked 12th among the most prevalent diseases in the world. Of course, this will be accompanied by a huge financial cost, which will lead to a great economic and social burden. For example, for dental treatment, About 60,000 children are under general anesthesia in England at an estimated cost of £27.6m, equivalent to running 3 secondary schools (7).

ECC leads to pain and destruction of tooth, it affects the oral and general health of children. As a result, there is an increased risk of permanent tooth decay in addition to eating, orthodontic and speech problems. In addition, the lives of parents can be affected (4).

Parental use of high doses of acetaminophen as an analgesic for early childhood caries may cause liver damage in young children (9).

Because early detection contributes to the success of treatment, there is a growing interest in biochemical indicators as an important tool in detecting disease and monitoring its progression. Therefore, it was necessary to study these indicators and their relationship to diseases affecting the mouth and teeth.

Salivary (8-Hydroxy-2' -Deoxyguanosine) (8-OHdG) may be used as a suitable biomarker in the diagnosis and monitoring of periodontitis disease. It is an important indicator of Deoxyribonucleic Acid (DNA) damage due to oxidation of guanzine by Reactive Oxygen Species **(**ROS) (15).

In another study, (8-OHdG) levels decreased in gingival sulcus fluid samples after periodontitis treatment (23). This leads to studies exploring their importance in dentistry.

In addition, the frequent use of C-Reactive Protein (CRP) as a biomarker in routine clinical applications makes its use in dentistry more acceptable. There is no doubt about the link between salivary CRP levels and tooth caries, according to numerous research. One of the studies showed that the serum CRP level increased in 30% of the dental caries patients who participated in the study. Considering how tooth caries and CRP levels are related, and considering that CRP level is one of the risk factors for cardiovascular diseases, dental caries may be one of the indirect factors (33).

Saliva contains many biomarkers, for example Interleukin-21 (IL-21) levels have an important role in causing cellular damage. This damage is one of the hallmarks of periodontitis. Therefore, IL-21 level was accepted is a crucial diagnostic biomarker in the detection of periodontitis (48). In this study, we focused on the relationship of serum IL-21 with early childhood dental caries.

Recently, there has been increased interest in using oxidative stress as an important diagnostic marker for many diseases. Therefore, the bulk of this study is To assess the significance of oxidative stress in the context of oral and dental diseases in children. Oxidative stress disorders may be responsible for dental caries, as it is believed to explain one of the mechanisms of periodontitis and dental caries (58).

One of the studies showed an increase in the level of Malondialdehyde (MDA), which is considered a sign of oxidative stress. It explained the high level of Malondialdehyde resulting from dental caries, which led to a decrease in the antioxidant level and thus the development of caries (60).

Additionally, according to one study, an increase in salivary Total Antioxidant capacity (TAC) level was found in children with dental caries compared to healthy children. This increase in level may be due to a compensatory mechanism against oxidative stress, according to the same study (88).

Based on the foregoing, a non-invasive methods are needed to monitor the progression of dental caries. This makes it appropriate to use biochemical indicators that prove their relationship with dental caries. It is necessary to search for more effective and less invasive methods for early detection of dental caries in children, which allows for fewer treatment procedures.

2. LITERATURE REVIEW

2.1. Early Childhood Caries

2.1.1. Definition of Dental Caries

In addition to their aesthetic role, teeth also play an important role in the process of digesting food. Therefore, they must be protected and continue to be present in the mouth. But it is exposed to things that affect its existence. One of the most threatening causes is dental caries.

In general, dental caries is an infectious disease that affects the teeth and causes local damage to the teeth. It is the gradual removal of mineralization in the teeth caused by bacterial acid, and it starts on the surface layer of the enamel. Its final effect breaks down the enamel and dentin, opening the way for bacteria to reach the pulp, which causes inflammation of the pulp and then the periapical tissues, which is one of the most common causes of toothache (1).

Apart from the pain it causes, the psychological effect it leaves, and the economic cost it gives to the patient, it is the most common cause of tooth loss(6).

2.1.2. Definition of Early Childhood Caries (ECC)

Early childhood caries (ECC), as defined by the American Academy of Pediatric Dentistry in 2003, is the presence of one or more decayed (non-cavitated or cavitated), missing (due to caries), or filled tooth surfaces in any primary tooth of a child under the age of six (7). The mother should be keen to provide a clean environment in the child's mouth by cleaning and sterilizing everything that touches his mouth, including bottles, tooth scratchers, toys, food containers, and even hands and fingers. Which can be painful for the child and subsequently affect the eruption and growth of the teeth. At least two to three minutes of brushing twice a day should be done by caregivers when the first tooth appears. A "pea-sized" tube of fluoride toothpaste is good for children and usually contains no more than 500 ppm of fluoride. Mothers should try to accustom their children to dental cleaning. It should make them more aware of how important it is to brush their teeth every day (4).

2.1.3. Consequences of Early Childhood Caries

World Health Organization (WHO) states that early childhood caries cause dental problems by directly reflecting on the child's lifestyle, physical and psychological health (depression, low self-esteem, anxiety), sleep disorders, malnutrition, shyness in smiling, digestive system, and chewing disorders, and delays in growth and development compared to peers (7).

2.1.4. Epidemiology

Early childhood caries ranked 12th of the most prevalent illnesses worldwide. In the United States, the prevalence rate in children aged 2-11 is 40%. It is considered one of the most widespread illnesses affecting children worldwide. In 2015, the number of infected children reached 560 million children of all ages (4,7).

2.1.5. Etiology of Early Childhood Caries

The mineralized tissues of the tooth are dissolved by organic acids generated by tooth dwelling bacteria. Dental caries is a complex illness including relationships between three main factors: the host, the microbiota, and the diet, especially fermentable carbohydrates. The infection can reach to the root apex, causing abscesses. For caries to develop, all three conditions must be favorable to the starting of disease. Because caries is generally gradual and reversible in the early stages, a noticeable carious lesion needs the action of all three elements over a significant amount of time (1). Three main circles are used to illustrate these components in a diagram (Figure 2.1).

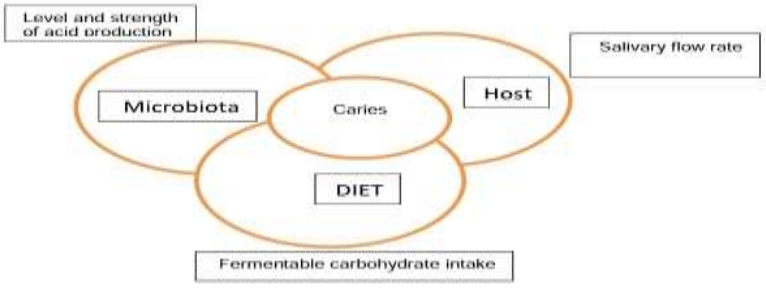


Figure 2.1 Causes of Dental Caries (1).

The physicochemical process of hydroxyapatite crystals being broken down by bacterial acids is known as demineralization (1). Both microbial colonization and the degree of prolonged low pH stimulus required for the disease are influenced by the amount of mineralization and architecture of tooth tissues. The figure shows examples of several forms of caries (Figure 2.2).

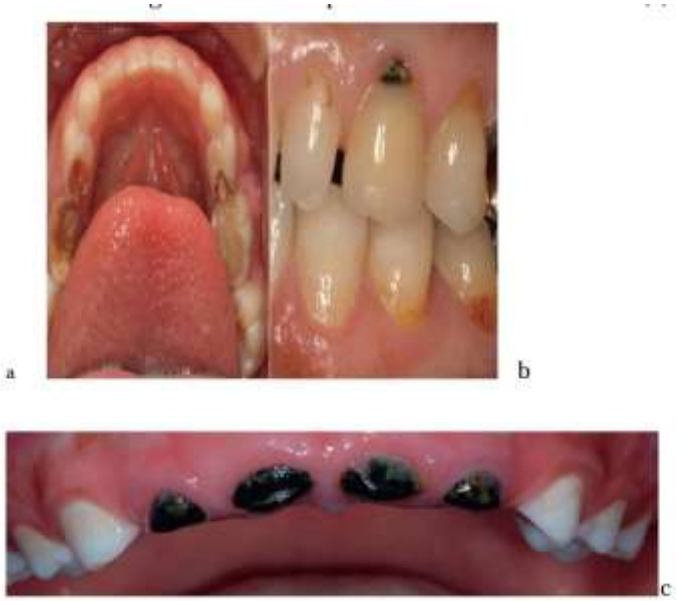


Figure 2.2. Examples of Various Forms of Caries (1).

Bacteria in the biofilm break down fermentable carbohydrates in the meal, producing organic acids that destroy the hydroxyapatite structure of teeth (3). Several oral bacteria may metabolize monosaccharides, resulting in enhanced acid generation and demineralization of the enamel. Streptococci and Actinomycetes dominate dental plaque on medically sound enamel in youngsters, and when carbohydrates are eaten, particularly sweet foods and beverages, the commensal plaque microbiota absorbs them and converts them to acids, primarily lactic acid, and when acid is produced, the pH shifts from roughly 7 (neutral) at about 5.5 (acidic). A decrease of highly cariogenic bacteria (Candida spp., Mutans Streptococci, and Lactobacilli) cannot be achieved if oral hygiene and dietary habits do not alter. Prevotella spp and Veillonella spp were shown to be the principal causes of caries rather than S.Mutans. Prevotella spp, Veillonella parvula, Veillonella atypical, Veillonella dispar, and V. parvula have all been identified as bacteria that are primarily related for the formation of ECC. They've been found to be microbial hazard factors, and bacteria, when combined with fungus,

can cause acid metabolism and virulence in microorganisms (4). The creation of organic acid (in the form of H^+) by bacteria eating carbohydrates leads plaque pH to drop, leading to the break down of HA into calcium ions, hydrogen phosphate ions, and water, which starts the demineralization process inside the enamel, and a thick coating of Calcium, Fluoride, and Phosphate accumulates on the surface during remineralization, providing increased resistance to subsequent demineralization (3). The ability of the fluoride anion to prevent bacterial development has long been known. The anion bonds to the enamel, generating Fluorohydroxyapatite, a more acid-resistant material. Fluoride ions have antibacterial properties because they block the enzyme Enolase making bacteria unable to flourish and increase tooth demineralization in the carious process (3). Caries is rife (Xerostomia) is a disorder in which salivary flow is decreased, resulting in widespread and severe carious diseases. Xerostomia can be caused by radiation therapy, which kills the salivary glands, or it may be inherited, as in the instance of Sjogren's syndrome, also Salivary flow is reduced by methamphetamine, which adds to widespread caries found in' meth mouth (1,5).

Xylitol is now well acknowledged as having anticarcinogenic qualities. Cranberries can also lower S. Mutans' glucosyltransferase activity, which reduces bacterial adhesion, on the another hand tea extracts are also suggested to reduce the amount of fermentable carbohydrates in the oral cavity by inhibiting salivary amylase activity (3).

The host's vulnerability to caries is influenced by a variety of elements in the oral environment. Caries are predominantly a juvenile illness since The tooth structure resists the loss of mineralization later (8).

2.2. 8-Hydroxy-2' -Deoxyguanosine (8-OHdG)

2.2.1. (8-OHdG) The Biomarker of Oxidative Stress and Carcinogenesis

Oxidative stress occurs when the equilibrium between the generation and concentration of (ROS and Reactive Nitrogen Species (RNS)) is disrupted. The antioxidants scavenge ROS and RNS, but at high enough concentrations, they can oxidize lipids, proteins, and DNA. (8-OHdG) is an indicator for DNA damage caused by oxidation. To employ 8-

hydroxy-2' -deoxyguanosine as a biomarker of oxidative stress excess production, background ranges must be determined (10).

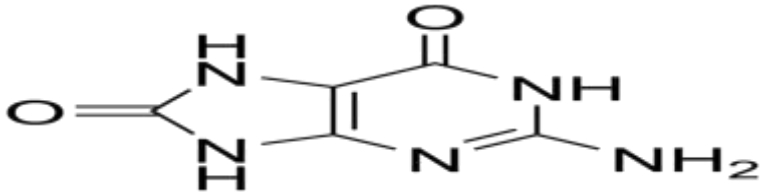


Figure 2.3. Structure of 8-OHdG (11)

One of the most prevalent types of oxidative lesions in nuclear and mitochondrial DNA caused by free radicals is (8-OHdG). And it has thus been commonly employed as a biomarker for oxidative stress and carcinogenesis. Urinary 8-OHdG has been shown to be an effective biomarker for assessing the risk of different malignancies and degenerative disorders in studies (11).

Because of the easiness with which 8-oxoguanine can be oxidized during extraction and the test technique, caution should be exercised while using it (12).

A crucial marker for assessing the impact of endogenous oxidative damage to DNA is 8-OHdG. And as a factor of carcinogenesis, as a result, it assesses the DNA damage caused by cancer causing chemicals such as cigarette and heavy metals, and in recent years, 8-OHdG has been used in several research, both as an indicator for assessing endogenous oxidative DNA damage and a risk factor for variety of disorders, including cancer (11).

When the hydroxyl radical reacts with the nucleobases of DNA like guanine, 8-OHdG is the result. Excessive amounts of 8-OHdG have been linked to disorders such as, cardiovascular disease and chronic obstructive pulmonary disease (13, 14).

2.2.2. (8-OHdG) and Oral Diseases

Periodontal disease is described as problems with the teeth' supporting tissues, such as the gingivae, periodontal ligament, and supporting alveolar bone, and is divided into gingivitis and periodontitis (15). Although there is no commonly accepted categorization of periodontal disease, clinical descriptors are used to describe the pace

of disease progression (acute or chronic), lesion distribution (generalized or localized) and the person's age group (prepubertal, juvenile or adult) (15).

Salivary analysis has become a crucial technique for assessing health and illness due to its source, serum-like components, and links with other organs (16).

Saliva includes molecular indicators that may be beneficial in the diagnosis, monitoring, and prognosis, of periodontal disease, according to medical experts, as the 8-hydroxideoxyguanosine (8-OHdG) - the product of guanosine oxidation by ROS - is an essential biomarker for DNA damage found in saliva (17,18).

Many studies have looked at the salivary 8-OHdG molecule in relation to periodontal tissue damage before and after therapy, and their findings demonstrate that this biomarker may be utilized to measure periodontal health of patient (19).

The discovery of salivary biomarkers and genotypes will allow for a more accurate diagnosis and a more effective prognosis. So, 8-OHdG level can be utilized to assess oro-dental health (15).

The res clear have a substantial connection between salivary concentrations of 8-OHdG and clinical markers of periodontal condition, according to recently published findings (20).

Salivary8-OHdG concentrations were observed to be higher in periodontal disorders in numerous additional investigations (21).

Salivary 8-OHdG has been linked to the presence of Porphyromonas gingivalis or a substantial mitochondrial DNA loss in gingival tissues in several investigations (22).

Several types of research looking into the blood 8-OHdG levels found that it may be altered by a variety of diseases. We may deduce that local 8-OHdG levels are strongly linked to periodontal disorders, with some implications for systemic diseases, but systemic 8-OHdG levels are more dependent on systemic conditions than on dental caries (23).

In patients with chronic periodontitis, plasma levels of (8-OHdG) rose, but these levels dropped after early periodontal therapy, according to studies. In groups of individuals with periodontitis significantly greater (8-OHdG) levels and lower overall antioxidant levels were found in the gingival blood (24).

The (8-OHdG) has been shown to be a biomarker of oxidative stress and has been connected to the etiology of autoimmune, inflammatory, and malignant diseases. Salivary and serum levels of (8-OHdG) have been investigated extensively in a variety of oral disorders, including Sjogren syndrome, oral malignancies, and periodontitis. There has been a link between periodontal bacteria and (8-OHdG), and early detection of salivary (8-OHdG) has been suggested as a helpful biomarker for properly diagnosing periodontitis and evaluating the success of treatment (25).

2.3. C-Reactive Protein

CRP is a kind of protein generated by the liver, when an injury, tissue death or infection occurs, it rises 1000 fold or more in blood level. That is linked to bodily inflammation, using a small blood sample CRP is applied to quantify inflammation symptoms, as well as to guide the treatment of bacterial infections and to monitor inflammatory processes in some autoimmune illnesses (26).

The liver produces CRP into the bloodstream to help the defenses organize when it is attacked by bacteria or other cellular invaders. Acute phase reaction refers to this early response (26).

CRP is a sensitive indicator of tissue damage and inflammation at the systemic level. It was the first acute-phase protein identified (27).

CRP is produced in many sites within the human body (Figure 2.4) (26). Human serum CRP is produced by activated monocyte products in Hep 3B cells. Addition, Non-hepatic cells such as neurons, Kupffer cells and lymphocytes generate it in small quantities (28).

CRP can be produced by epithelial cells in the respiratory tract and the renal epithelium under specific conditions (29).

Human coronary artery smooth muscle cells may also manufacture CRP when stimulated by inflammatory cytokines (30).

Also, that protein is also produced by the atherosclerotic lesions, alveolar macrophages, neurons and kidney (31).

There's also evidence that lipid peroxidation and infections like Cytomegalovirus (CMV) might set off a proinflammatory cytokine cascade that results in CRP release (32).

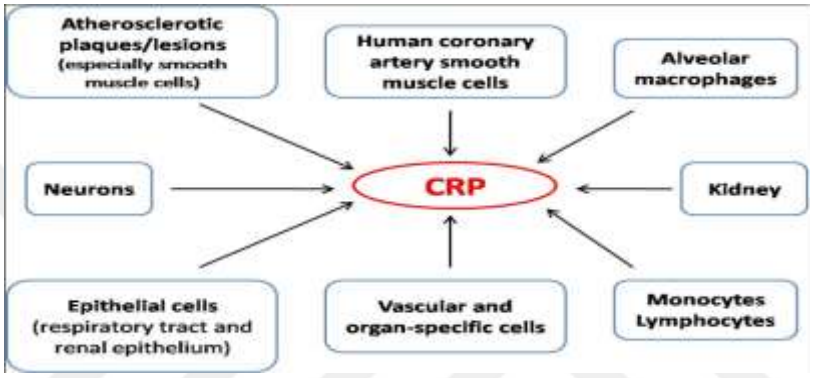


Figure 2.4. CRP Production in Different Parts of the Body Except the Liver (26)

C-reactive protein has been demonstrated to promote the production of IL-1α, IL-1β, Tumor Necrosis Factor Alpha (TNF-α) and IL-6 in blood mononuclear cells and alveolar macrophages (27).

2.3.1 Oral Diseases and CRP

Streptococcus mutans is a bacteria that is primarily responsible for tooth decay during dental caries. It is an acid-producing bacteria that causes dental caries by metabolizing the food in the host's teeth under favorable conditions (dry mouth, less saliva). This process normally takes 6 to 24 months (33).

If dental caries are allowed to progress without therapy, it will cause serious damage to the dental tissues, eventually leading to pulp and periodontal structures infection (33).

According to recent researches, dental caries is the host's innate immune response to bacterial infection, and enhanced C-reactive protein synthesis results from the release of inflammatory mediators from damaged tooth tissues (33).

According to some studies, increased CRP levels are associated with dental caries as well as preeclampsia and periodontitis (34).

A systemic inflammatory response brought on by periodontitis contributes to an elevated risk of cardiovascular disease. Considering that high CRP levels in plasma are a risk factor for cardiovascular disease and are based on the existence of an association between CRP level and periodontitis, this may explain the association between periodontal diseases and cardiovascular disease risk (72).

CRP levels typically rise for 1 to 4 days following inflammation then return to normal once these two variables have passed (33).

Untreated dental caries can result in chronic inflammation brought on by a lingering bacterial infection in the teeth, which raises the serum CRP level. Dental caries can therefore have a major impact on people's health and quality of life (33).

A high level of CRP, an inflammatory protein that is often present in large amounts during inflammation, has been associated with an elevated risk of cardiovascular disease (35).

In other research, regression analysis revealed that the levels of CRP in people with periodontitis were 1.72 times greater than those who didn't have periodontitis. several investigations on the link between periodontitis and CRP levels in the blood have been published (36,37).

Hepatocytes may be stimulated to generate CRP by inflammatory mediators produced by periodontitis. TNF-α, interleukin-6, interleukin-1 and are among the mediators engaged in this process (38). in this way, periodontal disease and dental caries can cause systemic inflammation and a considerable rise in CRP levels. According to the findings of certain research, cases with periodontitis have considerably greater levels of CRP than those that don't have periodontitis. These findings support

the concept that periodontitis is linked to increased production of this nonspecific inflammatory marker, which has far-reaching consequences for the periodontitis and dental caries patient (36).

In one previously looked at how systemic inflammation mediated the link between periodontitis and glycemic status in prior research. CRP, white blood cell count, and platelet-lymphocyte ratio caused 8%, 13%, and 16% of the link between severe periodontitis and diabetes, respectively, according to their findings (39).

For example, one research found that the presence of Porphyromonas gingivalis elevates CRP levels by about 20% (40).

Furthermore, the intensity of periodontitis may have a direct influence on CRP levels. CRP levels rise with the severity of periodontitis, according to one research, with instances with highly advanced and severe periodontitis having the highest CRP levels (41).

2.4. Interleukin-21

Interleukins (ILs) are a group of cytokines that were first identified in white blood cells. Cytokines are secreted proteins and signal molecules (leukocytes). Interleukins play a significant role in immune system function, and unusual deficits of a number of them have been identified, many of which are associated with autoimmune disorders or immunological insufficiency. CD4 helper T lymphocytes, monocytes, endothelial cells and macrophages produce the bulk of interleukins. B and T lymphocytes, as well as hematopoietic cells, develop and differentiate in response to interleukins (42).

2.4.1. Biological Importance of Interleukin-21

Interleukin-21 is a cytokine having a wide range of pleiotropic effects on lymphoid and myeloid cell development and activity. It belongs to the common gamma chain (c) family and is produced by a variety of immune cell types, such as natural killer (NK), T cells and T follicular helper (TFH) cells, being the main producers (43).

Interleukin-21 (IL-21), is mostly generated by activated T cells and is thought to play a major role in the transition between innate and acquired immunity. IL-21 has been demonstrated to influence the functioning of NK, T, and B cells, as well as myeloid cells. Furthermore, IL-21 has been shown to be a powerful antitumor agent, providing it an attractive option for new drug development. Different autoimmune and inflammatory disorders have been linked to IL-21 (44).

IL-21's Function in Lymphoid and Myeloid Cells

The effects of Interleukin-21 are dependent on the cell type as well as the differentiation and activation state of the cells. It's even been proposed that IL 21 plays a role in the transition from innate to adaptive immunity, owing to its capacity to restrict the length of NK cell activation and increase effector T-cell activities (45).

Infections Caused by Bacteria and Viruses

In Schistosoma mansoni–infected mice treated with soluble IL-21R-Fc, granulomatous inflammation, and liver fibrosis were reduced. This shows that, in addition to its key involvement in Th2-driven inflammation, IL-21R plays a major role in the formation of pathogen-induced Th2 responses as a significant amplifier of alternative macrophage activation (46).

Biopsy samples and isolated mucosal $CD3^{+}$ T cells from Helicobacter pylori-infected individuals showed higher levels of this cytokine (47).

After the identification of IL-21R and IL-21, this cytokine has been credited with a wide range of activities. In the control and generation of immunoglobulin, as well as the regulation and activation of NK, T, and B cells, IL-21 plays a key role. It has been shown that IL-21 inhibits the maturation of dendritic cells, works as an amplifier of alternative macrophage activation, and indirectly stimulates neutrophils, therefore its actions are not restricted to the lymphoid compartment, also can used in immunotherapy. This cytokine's enhanced expression has also been linked to autoimmune and inflammatory illnesses like rheumatoid arthritis as well as Crohn's disease (44).

2.4.2. Dental Caries and Interleukin-21

There is evidence that interleukin-21 contributes to the development of Th17 cells, which have been implicated in the pathogenesis of periodontitis and dental caries (48).

Numerous cytokines have been investigated in chronic periodontitis and their role in the degeneration of periodontal tissue, including interleukin-21 (49).

According to another study, an increase in salivary levels of IL-13, IL-4 and IL-2 is considered as salivary biomarkers in the dental caries process (74).

IL-6 and IL-8 levels were also increased in pediatric saliva in a study on the association of cytokines with dental caries in children. However, IL-8 turned out to be more closely associated with caries (75).

Another study was not limited to examining the salivary cytokines levels in the children with ECC, but also in their mothers' saliva. Children with early childhood caries showed an increase in the salivary IL-6 level compared to mothers of healthy children. In addition, the level of IL-6 was increased in early childhood caries pediatric saliva, doubled compared to the healthy Children (76).

In recent years research on IL-21, a newly discovered cytokine, has drawn attention (50).

According to a research, activated T cells release interleukin-21, which has the capacity to affect different immune system cells and influence infection resistance (49).

Chronic periodontitis is one of the most frequent inflammatory disorders in humans. The loss of periodontal tissues in sick individuals is known to be caused by a disproportionate adaptive response to microbial pathogens found in tooth plaque. The course of periodontal disease is influenced by both innate and adaptive immune responses. There are three categories of T helper cells: Th1, Th2, and Th17 cells. T cells have been shown to release inflammatory cytokines, which are responsible for periodontitis symptoms and consequences (52).

The role of Th17 cells in periodontitis and other systemic infectious diseases has been the subject of research recently. IL-21 contributes to the production of Th17 cells, which are important in the periodontitis disease process (53).

The majority of these studies discovered that those with chronic periodontitis had much greater IL-21 concentrations than healthy individuals, and numerous researchers found that IL-21 concentrations were strongly linked with periodontal characteristics (49).

One of the studies evaluated serum and salivary IL-21 levels using the Enzyme-Linked ImmunoSorbent Assay (ELISA) technique. It discovered a difference in serum and salivary IL-21 levels between periodontally healthy and chronic periodontitis patients. Many diagnostic indicators in saliva have been suggested and employed as a dental caries periodontal pathology analytical assistance. Early identification and treatment lessen the severity of the condition and its potential complications (48).

2.5. Oxidative Stress

The imbalance between reactive species generation and antioxidant defense is referred to as oxidative stress. A change in the pro-oxidant antioxidant balances in favor of the former, resulting in significant damage" is what oxidative stress is defined as (77). The death of a cell is caused by oxidative stress. ROS and RNS, are formed during metabolic and immune system activity in all organisms, including humans (54).

The body has a well-balanced system controlled by DNA, Proteins, Lipids and Carbohydrates and when these are destroyed by reactive oxygen species, it disrupts the metabolic condition, growth, and development of the organism's cells, leading to significant sickness (54).

ROS and RNS formation cause oxidative stress, which destroys nitrogenous bases as well as strand breaks in DNA such as hydrogen peroxide (H_2O_2), hydroxyl radical ($OH^{\bullet}$), and superoxide radical (O^{-2}). The hydrogen peroxide is notable because it crosses the membrane easily, has a prolonged half-life (1 minute), and is not segregated inside the cell (54).

Hydrogen peroxide, a highly reactive ROS created during oxidative stress, damages nucleic acids, proteins, carbohydrates, and lipids (54).

According to the existence of transition metals such as Cu^{+} and Fe^{++} (by the Fenton reaction), H_2O_2 combines with biomolecules to generate the extremely reactive hydroxyl radical, which is the most powerful oxidizing agent among all ROS, and RNS destroying carbohydrates, lipids, proteins, and DNA (54).

$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^{-} + HO^{\bullet}$ (Fenton's reaction) (54).

When the level of ROS rises above a certain threshold, it aids biological functions like phagocytosis, apoptosis, necrosis, and pathogen defense. In an oxidation reaction, numerous enzymes (peroxidase) use H_2O_2 as a substrate, resulting in the synthesis of complex organic molecules in the organism (56).

However, oxidative stress occurs when the quantity of ROS exceeds a particular threshold, causing damage to DNA, lipids, proteins, as well as carbohydrates. Various studies show that oxidative stress contributes to the formation or worsening of human illnesses like Alzheimer's disease, ulcerative colitis, Parkinson's dise, Atherosclerosis ,non-ulcer dyspepsia, major depression, Cancer, diabetic nephropathy, alcohol induced liver disease, end stage renal disease, mild cognitive impairment, Cardiovascular disease, neural disorders and aging (55).

In humans, the body has a defense mechanism that neutralizes the ROS and RNS effect. Antioxidants and endogenous antioxidants (such as Catalase and superoxide dismutase (SOD)), small proteins and molecules like glutathione, etc.) are the main defensive agents against ROS and RNS (54).

ROS are produced due to normal metabolism, drugs, aging, chemical, inflammation, radiation, and so on (Figure 2.5) (54).

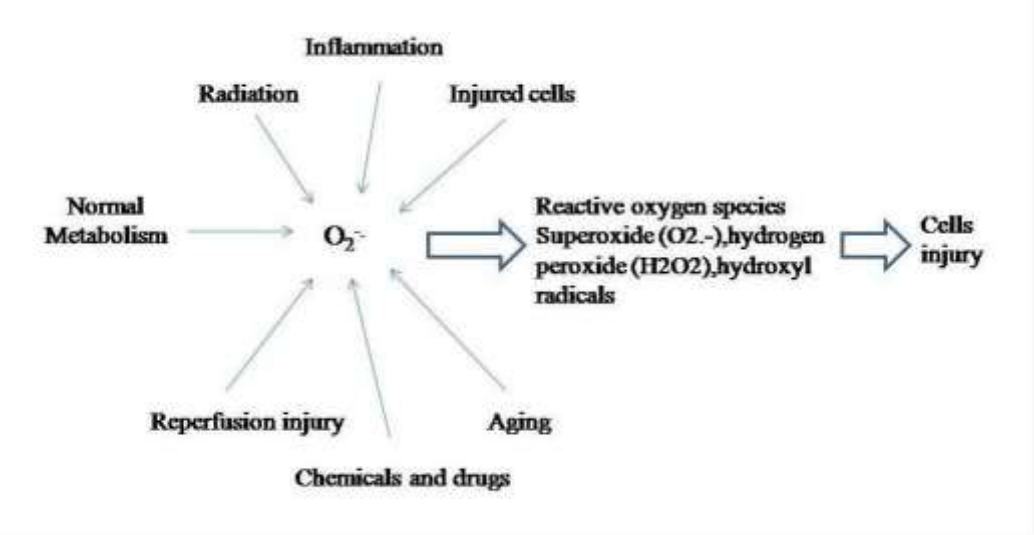


Figure 2.5. Formation of Reactive Oxygen Species (54)

2.5.1. Biomarkers of Oxidative Stress

The biomarkers that may be used to quantify oxidative stress have piqued attention since the proper evaluation of this stress is required for the examination of a variety of clinical disorders as well as the assessment of treatment efficacy, and from a clinical viewpoint, determining the amount of oxidative stress through biomarkers is intriguing. Although the indicators present in blood, urine, and other biological fluids may give diagnostic information, it would be ideal if organs and tissues suffering from oxidative stress could be viewed in the same way as CT scanning and MR imaging can. Attempts to employ electron spin resonance techniques for this purpose have been undertaken in recent years, but it will take time before such approaches can be used by people (57).

2.5.2. Dental Caries and Oxidative Stress

A pathological disease known as caries is marked by the demineralization brought on by the acids that bacteria in dental plaque produce as a result of the metabolism of sugar. Disorders in local and/or global oxidative stress parameters are thought to be one of the causes of the etiopathogenesis of periodontitis and dental caries, and the connection between oxidative stress indicators and oral disorders has been proven in several clinical trials (58).

The oral cavity is highly sensitive to reactive oxygen and nitrogen species, and under normal conditions, oxidative processes are catalyzed by NADPH oxidase or xanthine oxidase, as well as those produced during cellular respiration in the mitochondrial respiratory chain, are sources of ROS and RNS in the mouth (59).

Moreover, periodontal inflammation is one of the most major ROS generators in the mouth.

Additional resources include:

- Dental treatment (ultrasound, ozone, laser light, and ultraviolet light).
- Xenobiotics (ethanol and drugs).
- Food (high-protein high-fat, or high-sucrose diet).
- Dental materials (fluorides and dental composites).
- Air pollution, alcohol consumption, and smoking (59).

Saliva's ability to combat oxidative stress is due to the presence of low-molecular-weight antioxidants such as reduced glutathione (GSH), melatonin, ceruloplasmin, ascorbic acid, lactoferrin, transferrin and uric acid (UA), as well as enzymatic antioxidants such as salivary superoxide dismutase (SOD),catalase (CAT), and peroxidase (Px). Many studies indicate the major role of oxidative stress in salivary gland dysfunction. So, it is a pathogenetic element in conditions like caries and periodontitis (60).

CAT and uric acid are two indicators of oxidative stress. The first evaluates the total antioxidants in saliva, while the second is a key antioxidant defense component in saliva. Although there are few studies examining the association between caries and CAT, CAT may be used as a sensitive and accurate marker to detect antioxidant levels in biological samples and detect changes in oxidative stress. As a result, CAT and uric acid are useful diagnostic indicators for evaluating a variety of oral health issues (61).

Antioxidants are divided into three categories: chain-breaking, preventive, and enzymes, and they regulate ROS. For a healthy aerobic existence, antioxidant defense mechanisms eliminate free radicals (60).

The antioxidant system in saliva is one of several defense systems, and disease,inflammation or infection can affect salivary levels of antioxidant. For example, peroxidase works by regulating oral microorganisms to prevent dental caries and periodontal disease. Tissue damage and lipid peroxidation result from oxidative stress. The end result of lipid peroxidation is MDA (Malondialdehyde) (60).

A strong correlation between periodontal disease and oxidative stress has been found in numerous investigations on MDA in saliva in periodontitis (19).

Oxidative stress raises lipid peroxidation levels, and an disequilibrium between antioxidant capacity and oxidative stress may contribute to periodontitis etiology (62). Furthermore, periodontitis is associated with a reduction in overall antioxidant capacity (60).

When severe ECC left untreated, it progresses quickly. Early detection of ECC leads to therapies that are less intrusive and stressful (63).

Because of its non-invasive and safe collection, saliva is used as a biological fluid instead of plasma samples in the diagnosis and monitoring of systemic disorders and oral diseases (63).

In this regard, it is well documented that children with severe ECC have greater protein concentrations in their saliva than children who don't have caries. Because oxidative stress indicators are persistent in saliva, they may be discovered at measurable amounts there. These salivary biomarkers levels represent particular oxidation processes linked with periodontitis and dental caries (64).

In contrast, salivary superoxide dismutase (SOD) and uric acid (UA) levels in children with severe early childhood caries are much greater than in children without ECC. Showing that higher enzymatic and nonenzymatic antioxidant system activity might result in decreased oxidative damage in the saliva of individuals with dental caries (65).

Decreased glutathione (GSH) concentrations in the saliva of individuals with active dental caries, on the other hand, may suggest a decline in antioxidant capacity (66).

These contradicting findings suggest that aetiology and disease development might influence oxidative damage and antioxidant status indicators (63).

Salivary oxidative stress biomarkers were examined in one study after samples were taken for 30 days in a row and their concentrations varied widely. The salivary levels of oxidative stress indicators might fluctuate owing to local situations rather than systemic ones, one of the primary sources of ROS in the mouth is immune processes associated to periodontal disease. Oxidative stress in the mouth can also be caused by food intake, smoking, alcohol and dental treatment. As a result, the trustworthiness of saliva samples as oxidative stress indicators may be limited (67).

Oxidative stress is measured by lipid peroxidation products, DNA oxidation products, and oxidized proteins. Another method of determining redox state is to measure different antioxidants or total antioxidant status. However, using many biomarkers rather than one parameter gives more results and allows for a better understanding of the pathogenesis. Using the metabolomics technique, oxidative stress indicators were revealed to be one of the most discriminative between periodontitis patients and healthy controls. This demonstrates that studying salivary oxidative stress indicators is worthwhile (64).

High sucrose levels were shown to block the dentinal fluid flow, As a result, teeth become vulnerable to bacterial acids. Three equations, Health, Disease, and Disease, are used to give a condensed explanation of how oxidative stress causes inflammation (68).

Although it is not entirely apparent how oxidative stress contributes to dental caries, this is a hot area of research. Investigating salivary oxidative stress in relation to oral health involved several studies on children and adults (64).

The antioxidant capabilities of saliva are the focus of the majority of salivary oxidative stress studies in connection to dental caries. The salivary TAC was tested in eighty children. Age, gender, and caries activity were used to separate the children into eight groups. They discovered that caries-affected children had a non-significantly greater TAC, which they ascribed to the increased protein content in children with caries. The exclusion was the group of 11–15-year-old females, where the caries active group had reduced total protein and antioxidant capacity (69).

Saliva contains oxidative stress indicators in detectable amounts, specific oxidation mechanisms linked to caries and periodontitis are shown in these salivary indicators (63).

They were gathered Caries-free (group A), early carious lesions (group B), moderate carious lesions (group C), and advanced carious lesions (group D) were divided into four groups (n = 30) based on caries severity: caries-free (group A), early carious lesions (group B), moderate carious lesions (group C), and advanced carious lesions (group D) (group D). The salivary biomarkers were determined: total proteins (TP), oxidative damage, superoxide dismutase (SOD)total antioxidant capacity (TAC);enzymatic antioxidant activity; and uric acid (UA) nonenzymatic antioxidant activity. As a consequence, salivary oxidative damage was reduced as TP, SOD, TAC, enzymatic antioxidant activity, and UA non-enzymatic antioxidant activity rose with caries severity. Higher caries severity was found to boost salivary antioxidant system activity, resulting in a decrease in salivary oxidative damage (63).

2.5.3 Oxidative Stress Index (OSI)

When the equilibrium between the production and concentration of reactive oxygen and nitrogen species (ROS and RNS) is disturbed, oxidative stress results. Even though the antioxidant system can get rid of ROS and RNS, at high enough concentrations, they can damage lipids, proteins, and DNA (10).

To assess oxidative balance, total oxidative status (TOS) and total antioxidant status (TAS) measures were used. Instead of evaluating various oxidant and antioxidant

molecules independently, these factors were used to reveal the overall impact of oxidative stress (89).

2.5.4 Total Oxidants Status (TOS)

Reactive oxygen species are made when the body's normal metabolism, age, chemicals, inflammation, drug use, radiation, and other things happen (54).

Different oxidant species' serum concentrations can be tested independently in lab settings, but the procedures are labor-intensive, expensive, and time-consuming. Since it is impractical to detect each oxidant molecule independently and because their oxidizing effects are additive, a sample's total oxidant status (TOS), also known as total peroxide (TP), serum oxidation activity (SOA), reactive oxygen metabolites (ROM), or another term, is determined (82).

2.5.5 Total Antioxidants Status (TAS)

Humans have a defense mechanism in place that counteracts the ROS and RNS effects. Antioxidants and endogenous antioxidants (such as catalase and superoxide dismutase (SOD)), tiny proteins like thioredoxin and glutaredoxin, and compounds like glutathione, among others, are the primary protective agents against ROS and RNS (54). A hazardous process is avoided or inhibited by antioxidant molecules. The amounts of various antioxidants in serum can be determined individually in laboratories, but the procedures are labor-intensive, expensive, and time-consuming. Since it is impractical to measure each antioxidant molecule separately and because their antioxidant effects are additive, a sample's total antioxidant capacity (TAC), also known as total antioxidant activity (TAA), total antioxidant power (TAOP), total antioxidant status (TAS), or total antioxidant response, is determined (81).

3. MATERIAL and METHODS

3.1. Formation of Working Groups

45 patients children with early childhood caries (30 males and 15 females) and 45 healthy caries-free children (33 males and 12 females) were included in this study. The ages of the children in the control and patient groups were under six years. The patients were selected among the individuals who applied to Harran University, Faculty of Dentistry, Department of Pediatric Dentistry, due to dental problems between (2020-2021). According to the difficulty in controlling these children during dental treatments, they will be under general anesthesia. The blood of our healthy control group was selected from the blood coming to the Biochemistry laboratory, and those who did not have any chronic or metabolic diseases were selected. Informed consent was obtained from the families of the children participating in the study.

This study was approved by the Ethics in Research Committee at Harran University Faculty of Medicine (Reference number: HRU-12.19.20).

3.2. Preparation of Samples

Blood samples were collected from patients with early childhood caries and individuals in the control group. Children's parents were asked to fast them for 12 hours, and blood samples (5 ml) were taken. The blood samples were centrifuged at 5000 rpm for 10 minutes, and the serums were separated. Serum samples were stored at - 80°C until laboratory analysis.

3.3. Materials and Methods

In our study, CRP, TAS, TOS, IL-21 and 8-OHdG levels were measured in serums by using the devices used in the Biochemistry Laboratory of Harran University Research and Application Hospital.

- Atellica CH Wide Range C-Reactive Protein (wrCRP) Analyzer.
- Derin dondurucu -80 °C (Thermo).
- Spektroflorometre (Thermo Fisher Scientific, Vantaa, Finland).

- Centrifuge (Hettich Universal 30 RF).
- Microplate Washer (Bio-Rad, France).

3.3.1. Measurement of CRP Level in Serum

C-reactive protein measurement is helpful in assessing the amount of damage to body tissues.

When determining the quantitative level of CRP concentration in human blood and plasma (lithium heparin) for in vitro diagnostic usage, the Atellica CH Wide Range C-Reactive Protein (wrCRP) test was utilized. The Atellica CH wrCRP assay latex reagent consists of a suspension of uniform polystyrene latex particles coated with an anti CRP antibody. When serum or plasma containing CRP is mixed with the latex reagent, coalescence occurs, resulting in increased turbidity. This turbidity is measured at 571 nm. The CRP concentration in serum or plasma is determined from a calibration curve generated with the calibrators.

The system automatically performs the following steps:

1. For serum/plasma, dispense 50 µl of primary sample and 200 µl of Atellica CH Diluent into a dilution cuvette.
2. Dispense 50 µl of Reagent 1 into a reaction cuvette.
3. Dispense 10 µl of prediluted sample into a reaction cuvette.
4. Dispense 50 µl of Reagent 2 into a reaction cuvette.
5. Mix and incubate the mixture at 37°C.
6. Measures absorbance after reagent 2 is added.
7. Reports the results.

3.3.2. Determination of Total Antioxidants Status (TAS) Level

After 12 hours of fasting, 5 ml venous bloods was collected and stored at -80 °C after centrifugation 5000 rpm for 10 minutes.

It was measured by spectrophotometric method using the appropriate test kit (Rel Assay Diagnostics, Şehitkamil, Gaziantep, Turkey). It is a fully automated method

developed by Erel (81). Trolox was used as a calibrator, a water-soluble analogue of vitamin E. The results were represented as mmol Trolox equivalents/litre.

Reagents

Reagent 1: o-Dianisidine (10 mM) and $Fe(NH_4)_2(SO_4)_2$-$6H_2O$ (45 μM) were dissolved in Clark buffer solution (75 mM) (pH=1.8).

Reagent 2: Hydrogen peroxide (7.5 mM) is mixed in Clark buffer solution (75 mM) (pH=1.8).

Principle of Assay

The Fe^{+2}–o-dianisidine complex forms the OH radical via the Fenton reaction. At low pH, strong reactive oxygen species are reduced and react with the colorless O-dianisidine molecules to form yellow brown dianisidyl radicals. However, the antioxidants in the samples prevent the color formation by preventing these oxidation reactions. This reaction is measured spectrophotometrically in an automatic analyzer and the result is given .

3.3.3. Determination of Total Oxidants Status Level (TOS)

After 12 hours of fasting, 5 ml of venous blood was collected and centrifuged at 5000 rpm for 10 minutes and stored at -80 °C.

It was measured using the appropriate spectrophotometric method. The test kit (Rel Assay Diagnostics) developed by Erel was used (82). Hydrogen peroxide was used as Calibrator and results are expressed as μmol H_2O_2.equivalent/liter.

Reagents

Reagent 1: The main solution is prepared by dissolving (25 mM) of H_2SO_4 in (140 mM) of NaCl solution. It is prepared by first dissolving glycerol (10%) in the main solution and then dissolving (250 μM) of Xlenol orange in total volume.

Reagent 2: it is prepared by first dissolving(10 mM) of o-Dianisidine dihydrochloride and then (5mM) of ammonium ferrous sulfate were dissolved in the main solution.

Principle of Assay

The oxidants present in the sample oxidize the ferrous ion-o-dianisidine compound to ferric ion. Xylenol orange and ferric ions form a colored complex in acidic medium. The density of the color, which is related to the amount of oxidants in the sample, is measured.

3.3.4. Calculation of The Oxidative Stress Index

TOS and TAS results are presented as μmol H_2O_2 equivalents \L and μmol trolox equivalents \L, respectively. The ratio of TOS to TAS revealed the oxidative stress index (OSI), which was used as an indicator for total oxidative stress. The OSI value was calculated according to the formula below.

$$OSI = \frac{\text{TOS, } \mu\text{mol } H_2O_2 \text{ Equiv. / L.}}{\text{TAS, mmol trolox Equiv. / L. X 10}}$$

3.3.5. Determination of IL-21 Level

(IL-21) levels were examined by ELISA method. It was carried out according to the method reported by the manufacturer (BT LAB; Jiaxing, Zhejiang, China. Co, Ltd, Catalogue No: E10057Hu).

The principle of this Elisa assay is (Enzyme-Linked Immunosorbent Assay). A Human IL-21 antibody has been used to pre-coat the plate. When a sample antigen is supplied, the antibodies coated in the wells attach to it. Human antibodies that have been biotinylated are then introduced and to the antigen in the sample. The biotinylated antibody is then bound to by the addition of streptavidin When the substrate color attached HRP. Following incubation, a washing process removes any unbound streptavidin-HRP. Solution is added, the amount of human antigen determines how much will appear. At 450 nm, absorbance is measured after the addition of an acidic stop solution to the process halt it. This test will specifically identify each antigen in the human sample.

Standard Reconstitute the 120µl of the standard (1600ng/L) with 120ul of standard diluent to generate an 800ng/L standard stock solution. We allowed the standard to sit for 15 minutes with gentle agitation prior to making dilutions, then we Prepared duplicate standard points by serially diluting the standard stock solution (800ng/L) 1:2 with standard diluent to produce 400 ng/L, 200 ng/L, 100 ng/L and 50 ng/L solutions. Standard diluent serves as the zero standards (0 ng/ml).

we prepared the wash buffer by added concentrate 25 xs into deionized or distilled water to yield 500 ml of Ix Wash Buffer. And we mixed gently until the crystals have completely dissolved.

Procedure:

Before the study, all solutions present in the kit were brought to room temperature.

1. We added 50 µl of standard to the standard well.
2. 40ul of sample was added to the sample wells, and then 10ul of anti-IL-21 antibody was added to the sample.
3. 50µl of streptavidin-HRP Conjugate (SABC) working solution was added to standard wells and sample wells. Then it was mixed well.
4. Covered the plate with a concealer. The mixture was incubated at 37°C for 60 minutes.
5. The sealer was removed and the plate was washed 5 times with wash buffer. Wells were soaked with at least 0.35 ml of wash buffer for 30 seconds to 1 minute for each wash.
6. 50µL of substrate solution A was added to each well, and then 50µL of substrate solution B was added to each well. The plate was incubated for 10 min at 37 °C in the dark, coated with a fresh sealant.
7. Added 50µl of Stopping Solution to each well, the blue color will change to yellow immediately.
8. The optical density (OD value) of each well was determined using a microplate reader set to 450 nm within 10 minutes after adding the stop solution.

3.3.6. Measurement of 8-OHdG Level

The examination of (8-OHdG) levels was studied with the ELISA method. It was carried out according to the method reported by the manufacturer (BT LAB; ,Jiaxing,Zhejiang,China. Co, Ltd , Catalogue No: E1436Hu).

Principle

This kit is an enzyme-linked immunosorbent assay (ELISA). The plate is pre-coated with an (8-OHdG) antibody. (8-OHdG) in the blood sample is placed in the wells where it binds to the antibodies. The biotinylated (8-OHdG) antibody is then added to the sample and binds to (8-OHdG).

The biotinylated (8-OHdG) antibody then binds with Streptavidin-HRP. During a wash step after incubation, unbound Streptavidin-HRP was washed out. Substrate solution is added and the color develops in proportion to the amount of (8-OHdG) T. The reaction is stopped by adding an acidic stop solution and absorbance is measured at 450 nm.

The 120µl standard (128ng/ml) was diluted with 120µl of standard diluent to create a standard 64ng/ml standard stock solution. The standard was allowed to sit for 15 minutes with gentle mixing before dilution of the solution. We prepared binary standard points with standard diluent to produce solutions of 32ng \ml, 16ng \ml, 8ng \ml and 4Ng \ml. The standard diluent serves as zero standards (0 ng \ml). 20 ml of wash buffer concentrate 25x was placed in deionized or distilled water to obtain 500 ml of Wash Buffer IX. It was mixed gently until the crystals were completely dissolved.

Procedure:

1. All solutions available in the kit were brought to room temperature before the study. The test was carried out at room temperature. The steps specified in the kit were applied in the preparation of blood samples, standard solutions and reagents used.
2. We added 50 µl of standard to the standard well.
3. 40µl of sample was added to the sample wells, and then 10µl of anti-(8-OHdG) antibody was added to the sample wells.

4. 50µl of streptavidin-HRP Conjugate (SABC) working solution was added to standard wells and sample wells. Then it was mixed well.
5. Covered the plate with a concealer. The mixture was incubated at 37°C for 60 minutes.
6. The sealer was removed and the plate was washed 5 times with wash buffer. Wells were soaked with at least 0.35 ml of wash buffer for 30 seconds to 1 minute for each wash.
7. 50µL of substrate solution A was added to each well, and then 50µL of substrate solution B was added to each well. The plate was incubated for 10 min at 37 °C in the dark, coated with a fresh sealant.
8. Added 50µl of Stopping Solution to each well, the blue color will change to yellow immediately.
9. The optical density (OD value) of each well was determined using a microplate reader set to 450 nm within 10 minutes after adding the stop solution.

3.4. Statistical Analysis

For statistical analysis, we used the SPSS Windows version 23.0 package program. The conformity of the variables to the normal distribution was examined by histogram graphs and the Kolmogorov Smirnov/Shapiro-Wilk test. We used descriptive statistics (maximum, minimum, mean, and standard deviation) values for numerical variables. For the distributed variables between two groups, we used a t-test for the normal variables (parametric) in the groups, and for non-normal variables (nonparametric), we used the Mann-Whitney U test for statistical analysis, and the p-value ($P<0.05$) was considered statistically significant. While presenting the categorical variables, the frequency and percentage values of the variables were used. A Spearman Correlation Test was used in the analysis of the measurement data with each other.

4. RESULTS

The sample for the study was obtained from the (8-OHdG) (ng/ml), TAS (mmol/L), TOS (mmol/L), OSI (mmol/L), IL-21 (ng/L), and CRP (mg/dl) values of 90 individuals, 45 of whom were in the patient group and 45 of whom were in the control group, and the data were analyzed.

Table 4.1. Descriptive Statistics of Variables

	Minimum	Maximum	Median	Mean + std .Deviation
8-OHdG (ng/ml)	3.02	14.38	7.21	7.83±3.23
TAS (mmol/L)	0.36	2.05	1.21	1.25±0.21
TOS (mmol/L)	1.16	8.53	3.07	3.86±2.33
OSI (mmol/L)	0.01	0.08	0.03	0.03±0.02
IL-21 (ng/L)	64.44	182.56	132.39	127.38±43.06
CRP (mg/dl)	0.23	1.54	0.48	0.60±0.05

The study included 90 people in total: 45 patients in the in patient group and 45 healthy people in the control group. The mean of the (8-OHdG) measurement was 7.83 ng/mL. Its standard deviation is ±3.23. The minimum value of the (8-OHdG) measurement is 3.02 ng/mL, and the maximum is 14.38 ng/mL. The mean of the (TAS) measurement was determined at 1.25 mmol/L. Its standard deviation is ±0.21. The minimum value of the (TAS) measurement is 0.36 mmol/L and the maximum is 2.05 mmol/L. The mean of the (TOS) measurement was determined at 3.86 mmol/L. Its standard deviation is ±2.33. The minimum value of the (TOS) measurement is 1.16 mmol/L, and the maximum is 8.53 mmol/L (Table 4.1).

The mean of the (IL-21) measurement was found to be 127.38 ng/L. Its standard deviation is ±43.06. The minimum value of the (IL-21) measurement is 64.44 ng/L, while the maximum is 182.56 ng/L. The OSI measurement average was determined to be 0.03 mmol/L. Its standard deviation is ±0.02. The minimum value of the (OSI) measurement is 0.01 mg/dL, while the maximum is 0.08 mmol/L. The average CRP level was determined to be 0.60 mg/dL. Its standard deviation is ±0.05. The minimum

value of the (CRP) measurement is 0.23 mg/dL, while the maximum is 1.54 mg/dL (Table 4.1).

Table 4.2. Comparison of Serum Levels of 8-OHdG ,IL-21, CRP and Oxidative Stress Parameters in Early Childhood Caries Patients and the Control Groups

	Patient Group				Control Group				P
	Minimum	Maximum	Median	Mean + std. Deviation	Minimum	Maximum	Median	Mean + std. Deviation	
8-OHdG (ng/ml)	7.18	14.38	10.34	10.54±2.03	3.02	9.47	5.08	5.13±1.41	**<0.001**
TAS (mmol/L)	1.04	1.22	1.13	1.13±.04	0.36	2.05	1.38	1.38±0.24	**<0.001**
TOS (mmol/L)	3.31	8.53	5.66	5.90±1.47	1.16	2.83	1.60	1.82±0.51	**<0.001**
OSI (mmol/L)	0.03	0.08	0.05	0.05±0.01	0.01	0.05	0.01	0.01±0.01	**<0.001**
IL-21 (ng/L)	148.74	182.56	170.07	168.34±10.18	64.44	116.04	85.21	86.41±14.03	**<0.001**
CRP (mg/dl)	0.51	1.54	0.84	0.89±0.32	0.23	0.45	0.32	0.31±0.07	**<0.001**

Mann Whitney U Test

The mean serum 8-OHdG (ng/ml) levels in ECC patients (10.54±2.03) were higher than the mean serum 8-OHdG (ng/ml) levels (5.13±1.41) in the individuals in the Control Group and the difference between the patient and control groups was considered statistically significant ($p<0.001$). The 8-OHdG levels of the patient group were found to be a minimum of 7.18 and a maximum of 14.38. The 8-OHdG levels of the control group were found to be a minimum of 3.02 and a maximum of 9.47. In other words, the results showed a significantly higher level of serum 8-OHdG in the ECC patient group compared to the healthy control group (Table 4.2).

The mean serum TAS (mmol/L) levels in ECC patients (1.13±.04) were lower than the mean serum TAS (mmol/L) in the individuals in the Control Group (1.38±.24), and the difference between the patient and control groups was considered statistically significant ($p<0.001$). The TAS levels of the patient group were found to be a minimum

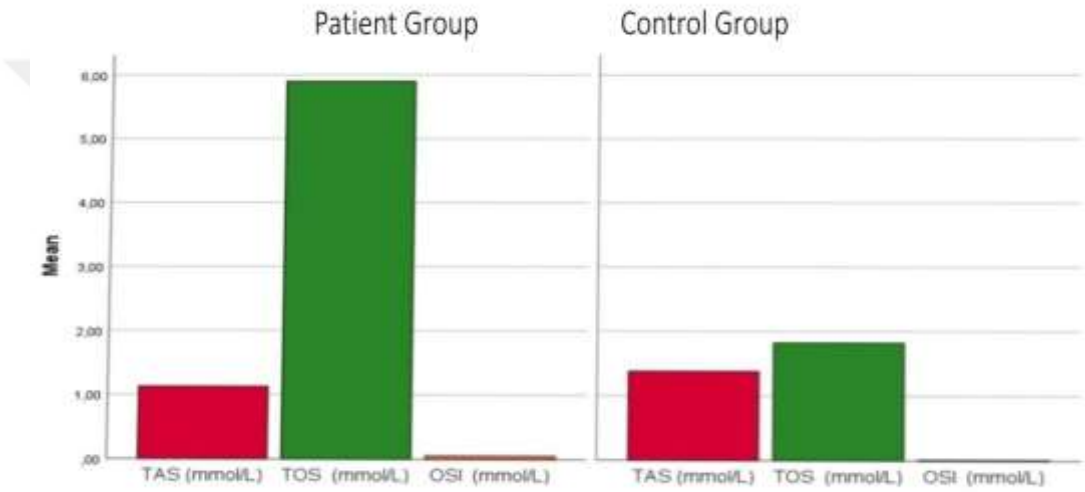
of 1.04 and a maximum of 1.22. The TAS levels of the control group were found to be a minimum of 0.36 and a maximum of 2.05. In other words, the results showed a significantly lower level of serum TAS in the ECC patient group compared to the healthy control group (Table 4.2).

The mean serum TOS (mmol/L) levels in ECC patients (5.90±1.47) were higher than the mean serum TOS (mmol/L) levels (1.82±0.51) in the individuals in the Control Group, and the difference between the patient and control groups was considered statistically significant ($p<0.001$). The TOS levels of the patient group were found to be a minimum of 3.31 and a maximum of 8.53. The TOS levels of the control group were found to be a minimum of 1.16 and a maximum of 2.83. In other words, the results showed a significantly higher level of serum TOS in the ECC patient group compared to the healthy control group (Table 4.2).

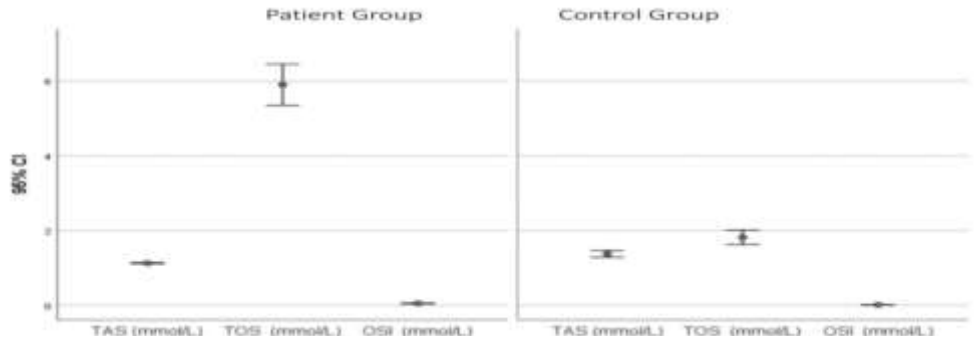
The mean OSI (mmol/L) values in ECC patients (0.05±0.01) were higher than the mean OSI (mmol/L) values (0.01±0.01) in the individuals in the Control Group, and the difference between the patient and control groups was considered statistically significant ($p<0.001$). The OSI values of the patient group were found to be a minimum of 0.03 and a maximum of 0.08. The OSI levels of the control group were found to be a minimum of 0.01 and a maximum of 0.05. In other words, the results showed a significantly higher value of OSI in the ECC patient group compared to the healthy control group (Table 4.2).

The mean serum IL-21 (ng/L) levels in ECC patients (168.34±10.18) were higher than the mean IL-21 (ng/L) levels in the individuals in the Control Group (86.41±14.03), and the difference between the patient and control groups was considered statistically significant ($p<0.001$). The IL-21 levels of the patient group were found to be a minimum of 148.74 and a maximum of 182.56. The IL-21 levels of the control group were found to be a minimum of 64.44 and a maximum of 116.04. In other words, the results showed a significantly higher level of serum IL-21 in the ECC patient group compared to the healthy control group (Table 4.2).

The mean serum CRP (mg/dl) levels in ECC patients (0.89±0.32) were higher than the mean CRP (mg/dl) levels in the individuals in the Control Group (0.31±0.07), and the difference between the patient and control groups was considered statistically significant ($p<0.001$). The CRP levels of the patient group were found to be a minimum of 0.51 and a maximum of 1.54. The CRP levels in the control group were found to be a minimum of 0.23 and a maximum of 0.45. In other words, the results showed a significantly higher level of serum CRP in the ECC patient group compared to the healthy control group (Table 4.2).



Graphic 4.1. Serum Oxidative Stress Parameter Levels in ECC Patients Compared to Levels in the Control Group



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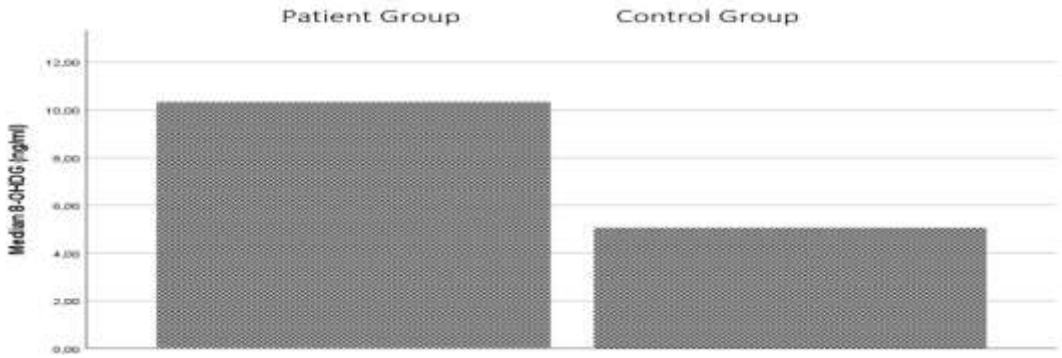
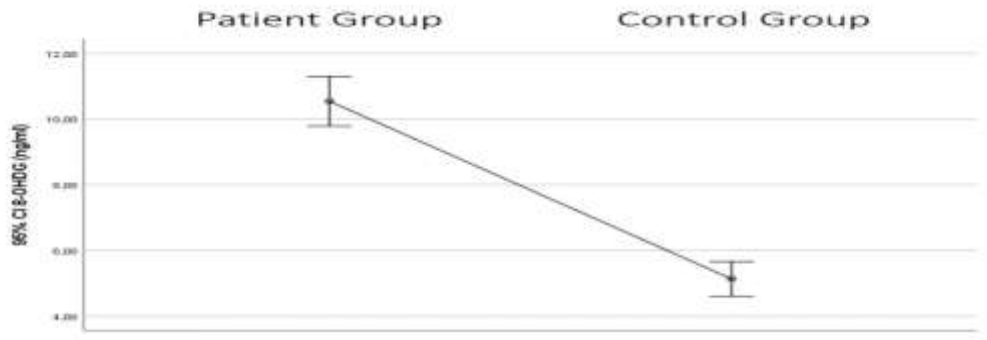
Graphic 4.2. The Changes in Mean Oxidative Stress Parameter Levels in the Patient and Control Groups (TAS.TOS.OSI).

The Mean seum TAS (mmol/L) levels in ECC patients (1.13±.04) were lower than the mean serum TAS (mmol/L) in the individuals in the Control Group (1.38±.24), and the difference between the patient and control groups was considered statistically significant (p<0.001). It was found that Mean serum TOS (mmol/L) levels in ECC patients (5.90±1.47) were higher than mean serum TOS (mmol/L) levels (1.82±0.51) in the individuals in the Control Group, and the difference between the patient and control groups was considered statistically significant (p<0.001). It was found that the Mean OSI (mmol/L) values in ECC patients (0.05±0.01) were higher than the mean OSI (mmol/L) values (0.01±0.01) in the individuals in the Control Group, and the difference between the patient and control groups was considered statistically significant (p<0.001) (Graphic .4.1).

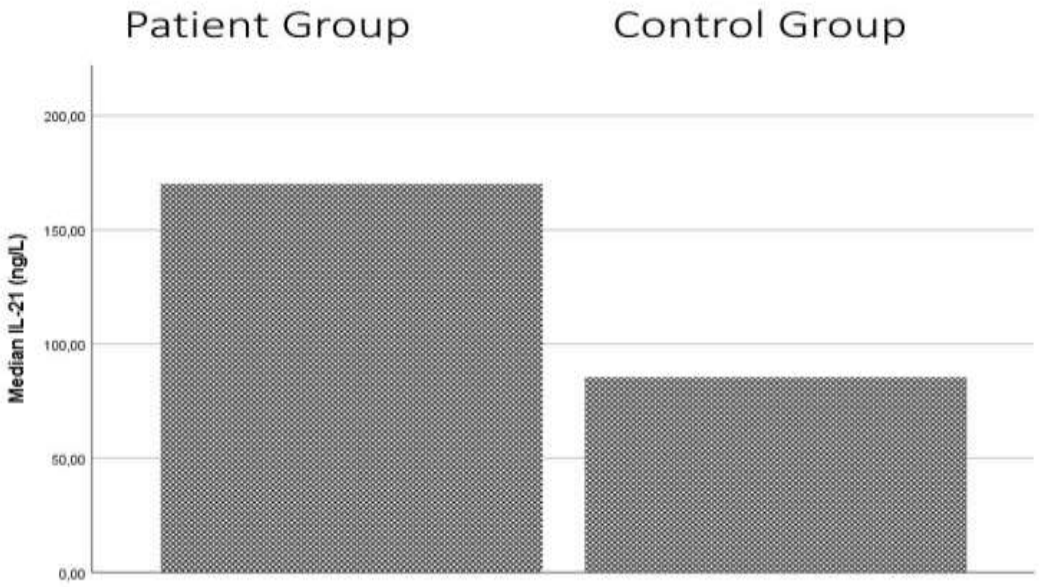


Graphic 4.3. Serum Levels of 8-OHdG Measured in ng/mL in the ECC Patients Group , Compared with Levels in the Control Group

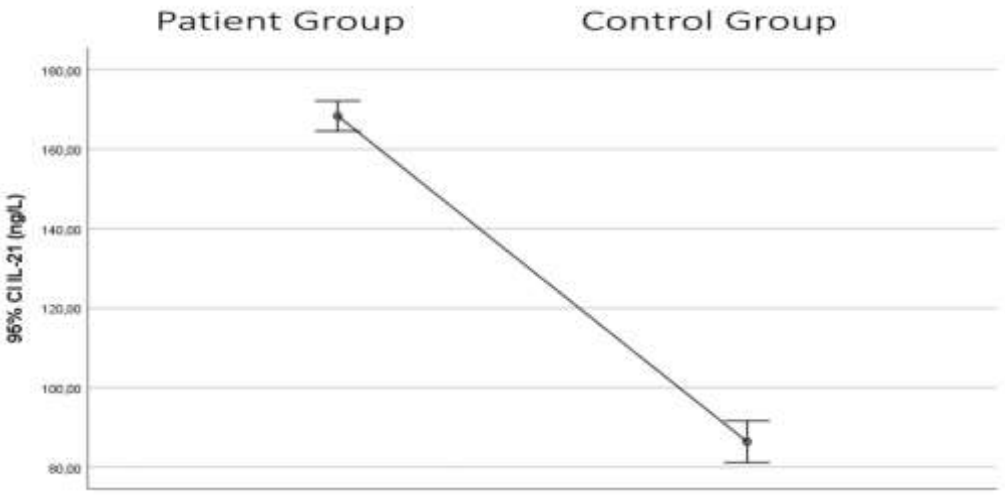


Graphic 4.4. The Change in Mean 8-OHdG Levels of ECC Patienst and Control Groups

The mean serum 8-OHdG (ng/ml) levels in ECC patients (10.54±2.03) were higher than the mean serum 8-OHdG (ng/ml) levels (5.13±1.41) in the individuals in the Control Group and the difference between the patient and control groups was considered statistically significant (p<0.001) (Graphic 4.3).



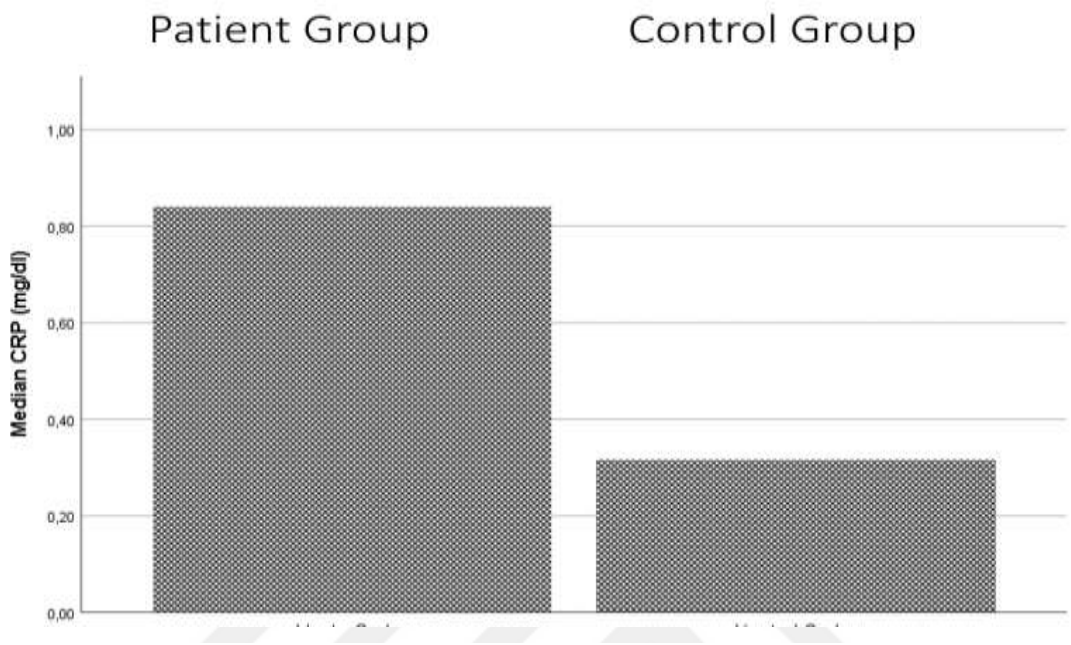
Graphic 4. 5. Serum levels of IL-21 Measured in ng/L in The ECC Patients Group, Compared with Levels in The Control Group.



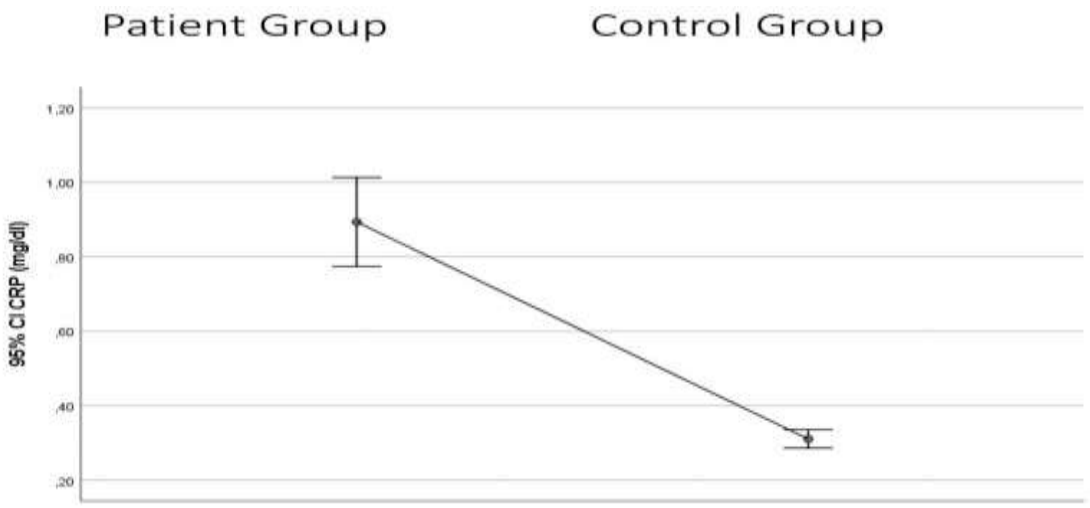
Graphic 4.6. Change in the Mean IL-21 Levels of ECC Patient and Control Groups.

The mean serum IL-21 (ng/L) levels in ECC patients (168.34±10.18) were higher than the mean IL-21 (ng/L) levels in the individuals in the Control Group

(86.41±14.03), and the difference between the patient and control groups was considered statistically significant (p<0.001) (Graphic 4.5).

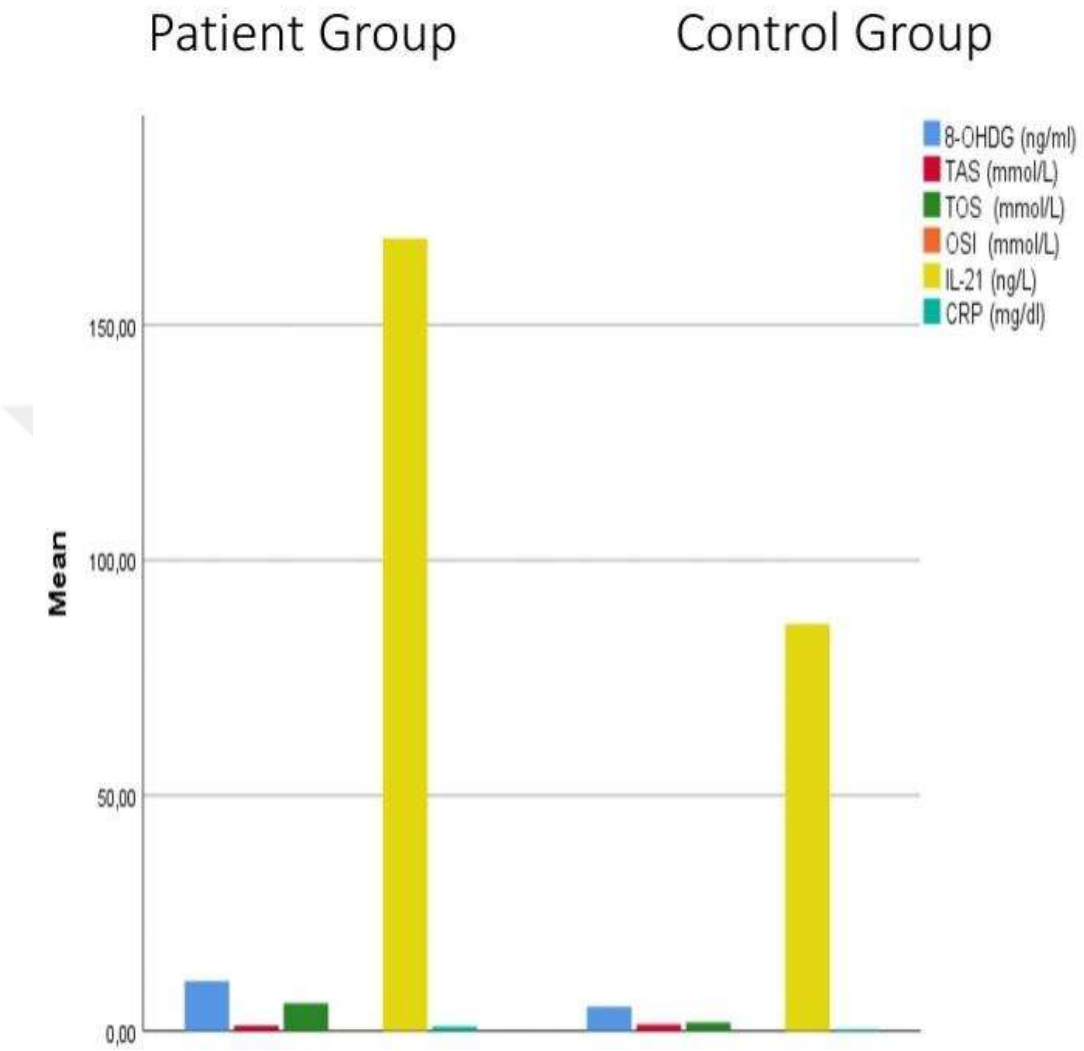


Graphic 4.7. Serum levels of CRP Measured in mg/dl in The ECC Patients Group, Compared with Levels in The Control Group.



Graphic .4.8. Change in the Mean CRP Levels of ECC Patient and Control Groups

The mean serum CRP (mg/dl) levels in ECC patients (0.89±0.07) were higher than the mean CRP (mg/dl) levels in the individuals in the Control Group (0.31±0.01), and the difference between the patient and control groups was considered statistically significant (p<0.001) (Graphic 4.7).



Graphic .4.9. CRP, IL-21, 8-OHdG, and Oxidative Stress Parameters in the ECC Patients Group Compared to the Control Group.

It was discovered that there were statistically significant differences between the patient and control groups' measurement levels of 8-OHdG, TAS, TOS, OSI, IL-21, and CRP. TAS measurement levels in the early childhood caries patient group were considerably lower than in the control group, despite the fact that 8-OHdG, TOS, OSI, IL-21, and CRP measurement levels in the early childhood caries patient group were significantly higher than in the control group (Graphic 4.9).

Table 4.3. Correlation of 8-OHdG, IL-21, CRP and Oxidative Stress Parameters in the Patient Group

		8-OHdG (ng/ml)	TAS (mmol/L)	TOS (mmol/L)	OSI (mmol/L)	IL-21 (ng/L)	CRP (mg/dl)
8-OHdG (ng/ml)	r	1.000	0.435	0.040	-0.013	0.057	-0.073
	p	.	**0.016**	0.834	0.945	0.764	0.701
TAS (mmol/L)	r	0.435*	1.000	0.026	-0.109	0.092	0.177
	p	0.016	.	0.891	0.566	0.629	0.350
TOS (mmol/L)	r	0.040	0.026	1.000	0.980	-0.214	-0.287
	p	0.834	0.891	.	**0.000**	0.257	0.124
OSI (mmol/L)	r	-0.013	-0.109	0.980**	1.000	-0.234	-0.320
	p	0.945	0.566	0.000	.	0.213	0.085
IL-21 (ng/L)	r	0.057	0.092	-0.214	-0.234	1.000	-0.171
	p	0.764	0.629	0.257	0.213	.	0.366
CRP (mg/dl)	r	-0.073	0.177	-0.287	-0.320	-0.171	1.000
	p	0.701	0.350	0.124	0.085	0.366	.

Spearman Correlation Test. *Correlation is significant at the 0.05 level. **Correlation is significant at the 0.01 level.

It was investigated whether there was a statistically significant correlation between the clinical measurements of 45 patients included in the study group, as a result of this research, while there is a moderately positive correlation between 8-OHdG and TAS (P = 0.016, R = 0.435) ($p<0.05$), there is a very strong positive correlation between TOS and OSI (P = 0.000, R = 0.980) ($p<0.01$) (Table 4.3).

A statistically significant correlation between 8-OHdG (ng/ml) values and TOS (mmol/L), OSI (mmol/L), IL-21 (ng/L), and CRP (mg/dl) values of individuals in the patient group was not determined ($p>0.05$).

It was determined that there was no statistically significant correlation between the TAS (mmol/L) values and the IL-21 (ng/L), TOS (mmol/L), OSI (mmol/L) and CRP (mg/dL) values of the individuals in the patient group ($p>0.05$).

It was determined that there was no statistically significant correlation between the TOS (mmol/L) values and the IL-21 (ng/L) and CRP (mg/dL) values of the individuals in the patient group ($p>0.05$).

It was determined that there was no statistically significant correlation between the OSI (mmol/L) values and the IL-21 (ng/L) and CRP (mg/dL) values of the individuals in the patient group ($p>0.05$).

It was determined that there was no statistically significant correlation between IL-21 (ng/L) and CRP (mg/dL) values of the individuals in the patient group (p>0.05).

Table 4.4. Correlation of 8-OHdG ,IL-21, CRP and Oxidative Stress Parameters in the Control Group

		8-OHdG (ng/ml)	TAS (mmol/L)	TOS (mmol/L)	OSI (mmol/L)	IL-21 (ng/L)	CRP (mg/dl)
8-OHdG (ng/ml)	r	1.000	-0.474	-0.108	-0.062	-0.002	0.181
	p	.	**0.008**	0.569	0.744	0.993	0.339
TAS (mmol/L)	r	-0.474**	1.000	-0.032	-0.311	0.161	-0.402
	p	0.008	.	0.866	0.095	0.396	**0.028**
TOS (mmol/L)	r	-0.108	-0.032	1.000	0.927	0.010	0.141
	p	0.569	0.866	.	**0.000**	0.959	0.458
OSI (mmol/L)	r	-0.062	-0.311	0.927**	1.000	-0.054	0.240
	p	0.744	0.095	0.000	.	0.776	0.202
IL-21 (ng/L)	r	-0.002	0.161	0.010	-0.054	1.000	-0.056
	p	0.993	0.396	0.959	0.776	.	0.769
CRP (mg/dl)	r	0.181	-0.402*	0.141	0.240	-0.056	1.000
	p	0.339	0.028	0.458	0.202	0.769	.

Spearman Correlation Test. *Correlation is significant at the 0.05 level. **Correlation is significant at the 0.01 level.

It was investigated whether there was a statistically significant correlation between the clinical measurements of the 45 children in the control group. As a result of the research, while there is a moderately negative correlation between (8-OHdG) and TAS (P = 0.008, R = -0.474), there is a very strong positive correlation between TOS and OSI (P=0.000, R= 0.927)(p<0.01). In addition, there is a moderate negative correlation between TAS and CRP (P=0.028, R= -0.402) (p<0.05) (Table 4.4).

Statistically significant correlation between 8-OHdG (ng/ml) values and TOS (mmol/L), OSI (mmol/L), IL-21 (ng/L), and CRP (mg/dl) values of individuals in the control group was not determined (p>0.05).

It was determined that there was no statistically significant correlation between TAS (mmol/L) values and TOS (mmol/L), OSI (mmol/L), and IL-21 (ng/L) values of the individuals in the control group ($p>0.05$).

It was determined that there was no statistically significant correlation between the TOS (mmol/L) values and the IL-21 (ng/L) and CRP (mg/dL) values of the individuals in the control group ($p>0.05$).

It was determined that there was no statistically significant correlation between the OSI (mmol/L) values and the IL-21 (ng/L) and CRP (mg/dL) values of the individuals in the control group ($p>0.05$).

It was determined that there was no statistically significant correlation between IL-21 (ng/L) values and 8- CRP (mg/dl) values of individuals in the control group ($p>0.05$).

5. DISCUSSION

Dental caries is defined as a bacterial infection of the tooth that produces acids leading to the demineralization of hard dental tissues. This loss weakens the tooth structure and allows irritants and infections to reach the pulp. Tooth decay is the most common cause of toothache and extractions. It is also the most common oral disease. Teeth are exposed to dental caries from the moment they appear in the oral cavity; therefore, dental caries can affect teeth at any age (1).

The imbalance between reactive species production and antioxidant defense is called oxidative stress. Biomarkers that can be used to measure oxidative stress have attracted attention. Appropriate assessment of this stress is essential for examining various clinical disorders and evaluating treatment efficacy. It is of interest to quantify oxidative stress through biomarkers (57).

Periodontal inflammation is one of the main sources of ROS in the mouth. ROS is produced by active granulocytes, monocytes, and macrophages as a defense against bacteria. Oral inflammation is a result of the inflammatory response with increased ROS and oxidative damage products in the oral tissue and saliva (59). Following bacterial antigen stimulation, ROS, or free radical molecular species, are produced by Polymorphonuclear leukocytes (PMNs) as a result of inflammatory tissue response mechanisms (84).

In the clinical studies conducted by Wei D. serum TOS levels were higher in the chronic periodontitis patient group than in the control group. Deterioration in the antioxidant defense system due to the overproduction of LPO products in inflammatory regions may be associated with higher levels of oxidative stress in saliva and serum in periodontitis patients (83).

In this study, we evaluated TOS levels in serum in children with ECC. The results showed higher TOS levels in the ECC group compared to the control group ($p< 0.001$). The relationship between dental plaque bacteria and the immune system may explain our results. Dental plaque bacteria stimulate the immune system to produce mediators that cause an inflammatory reaction. Granulocytes, neutrophils, and

monocytes consume a great deal of oxygen. This converts molecular oxygen to the superoxide radical anion, leading to the production of hydrogen peroxide. Hydrogen peroxide is toxic to bacteria and tissues. As a result, the total antioxidant status decreases (70).

TAC levels might serve as a marker for both gingival bleeding and dental caries. In a study by Salman et al. salivary TAC levels showed a significant decrease in samples with caries when compared to the caries-free group (90). Krawczyk et al. confirmed the relationship between TAS levels in saliva and dental caries. The level of TAS in the saliva of patients with dental caries indicates the probability of total antioxidant status influencing the course of dental caries. The use of these results and measures in preventive dentistry can play an important role in the monitoring and prevention of dental caries (70).

In this study, we evaluated TAS levels in serum in children with ECC. The results showed lower TAS levels in the ECC group compared to the control group ($p< 0.001$). The excessive generation of free radicals and consumption of antioxidants may be a factor in the decline in TAC levels. The immune system is unable to compensate for the consumption of antioxidants during the caries process. During the inflammatory process, the immune system neutralizes free radicals by consuming antioxidants (90). In a study conducted by Ahmadi-Motamayel et al. on the indicators of oxidative stress in the serum of patients with dental caries, they found an increase in oxidative stress in the study group compared with the control group. It was suggested that the increase in oxidative stress leads to a decrease in the level of antioxidants and the development of dental caries (60). The results of our study were consistent with this suggestion, as oxidative stress increased in our study and the level of antioxidants decreased, unlike in many other studies. It is possible to increase the level of antioxidants in the acute phase, but over time, the level of antioxidants may decrease as a result of their consumption (64).

During the dental caries process, antioxidant levels in the serum may decrease. This reduction is a result of MDA production, which leads to caries progression. MDA is a product of lipid peroxidation and an indicator of oxidative stress. A study conducted by Ahmadi-Motamayel et al. noted that patients with dental caries had increased serum

MDA levels compared to the healthy control group. It was also found in the study that the oxidative stress index increased in the group with dental caries compared to the healthy control group. It is not known whether MDA is the cause or effect of the disease. The local and systemic increase in salivary and serum MDA levels in the patient group with dental caries may be related to bacterial counts and the concentration of their products (60).

In this study, we evaluated OSI in serum in children with ECC. The results showed higher OSI in the ECC group compared to the control group ($p<0.001$). Bacteria and plaque are involved in the inflammatory process that leads to periodontal disease and dental caries; as a result, they may have an effect on the generation of oxidative stress indicators, though it is unclear how far this affects (90).

The functional and structural reconstructions that contribute to the onset of chronic periodontitis may be affected by the imbalance of reactive oxygen species and the antioxidant system. Chen M et al. found that the saliva of people with chronic periodontitis had a significant drop in total antioxidant capacity and a significant rise in malondialdehyde (MDA), nitric oxide, total oxidant status (TOS), and 8-hydroxy-deoxyguanosine levels (85).

The role of oxidative stress in the dental caries process is not fully understood despite extensive research on the relationship between oxidative stress and the pathogenesis of dental caries. So far, the source of free radicals is not known, although some studies have identified PMNs as a source of ROS and RNS, while other studies have indicated the role of bacterial plaque in the development of oxidative stress in the dental caries pathway (58).

Many of clinical studies indicated that periodontal inflammation-generated ROS spreaded into the blood, and gradually affecting other organs. Oral disorder due to OSI may be related to systemic disorders, such as pancreatic, gastric, cardiovascular and liver diseases. In additoin, more studies are needed about the genotoxic effects of dental caries, because children have a long lifespan so probability of accumulating mutations that eventually lead to carcinogenesis (92,93).

In our study, we found a very strong positive correlation between TOS and OSI in the patient and control groups ($p<0.05$). High total oxidant status and low total antioxidant status in serum are associated with high oxidative stress. In addition, our study showed a positive, statistically significant correlation between 8-OHdG and TAS levels in serum in patients with early childhood caries. An increase in oxidative stress-induced early oxidative damage on DNA molecules in periodontal tissues, as 8-OHdG is a good indicator of DNA damage, may stimulate the immune system to produce antioxidants to protect the periodontal tissues from free radicals (85, 90).

(8-OHdG) is one of the most common types of free radical-induced oxidative damage to nuclear and mitochondrial DNA. Therefore, it is often used as a biomarker of oxidative stress (11). Saliva biomarkers will allow for a more accurate diagnosis and a more effective prognosis. So, (8-OHdG) salivary level can be used to assess oro-dental health (15).

(8-OHdG) levels in saliva have been associated with periodontal disease in several studies. Paredes-Sánchez E et al. found in their results that (8-OHdG) levels in saliva were twice as high in patients with periodontitis. Salivary 8-OHdG levels that are higher during periodontal inflammation reflect enhanced oxygen radical activity (78). Higher salivary (8-OHdG) and MDA levels and lower salivary antioxidant activities reflect increased oxygen radical activity during periodontal inflammation (19).

Veljovi T et al. investigated 8-OHdG levels in periodontitis patients' saliva and plasma. The results showed a significant increase in the level of (8-OHdG) in saliva in periodontitis patients compared to the control group; this level decreased after periodontitis treatment (79). High levels of (8-OHdG) in saliva may indicate a higher index of oxidative stress due to periodontitis. Effective treatment of periodontitis reduces the level of (8-OHdG) in saliva (80).

There are no studies that are quite similar to ours to compare our findings to theirs, but our findings agree with many studies that looked at 8-OHdG in saliva and its relationship to periodontal diseases. In this study, we evaluated 8-OHdG levels in serum in children with ECC. Our results showed higher 8-OHdG levels in the ECC group compared to the control group ($p<0.001$). Since our study indicated an increase in the

oxidative stress index, an increase in the level of oxidants, and a decrease in antioxidants, we suggest that a high index of oxidative stress indicates damage to DNA molecules in the presence of pathogenic bacterial plaque (85). Higher salivary (8-OHdG) reflect increased oxygen radical activity during periodontal inflammation (19).

There is a positive correlation between clinical and microbiological markers of periodontal diseases and caries (87). The interaction between pathogenic bacteria and the host's immune response leads to an increase in cellular expression and immune activity in periodontal tissues. Polymorphonuclear leukocytes (PMN) are the primary mediators of the host response to pathogens induced by bacterial antigens (19).

CRP, a type of protein produced by the liver, is elevated in the blood when an injury, inflammation, or tissue death occurs. C-reactive protein (CRP) is an acute-phase protein belonging to the pentraxin protein family. This applies to a small C-reactive protein blood sample to measure symptoms of inflammation and also to guide the treatment of bacterial infections and monitor inflammatory processes in some autoimmune diseases (26).

There are many studies showing the relationship between CRP levels and oral diseases. An increase in CRP levels in serum was observed in periodontitis patients compared to the healthy group. The severity of the disease determines how much CRP levels rise in patients with periodontitis. Additionally, infections with subgingival organisms, which are frequently linked to periodontal disease, are associated with higher levels of CRP (72). Numerous studies have demonstrated a relationship between high serum CRP levels and chronic periodontitis. From a biological point of view, Periodontitis causes the production of inflammatory mediators (IL-1, IL-6, and TNF-α), which stimulates the hepatocytes to generate CRP. Therefore, we can find high levels of CRP in the serum if the patient has periodontitis (71).

There are not many studies that have examined the level of CRP in serum and its relationship with dental caries. The results of our study matched the results of the research conducted by Alanazi AF et al. who found that the level of CRP in the serum increased in 30% of patients with dental caries (33). In this study, we evaluated CRP levels in serum in children with ECC. The results showed higher CRP levels in the ECC

group compared to the control group ($p < 0.001$). In contrast to the results of our study, Doğan MS et al. did not record any association between early childhood caries and serum C-reactive protein levels in their study of the relationship between the dental caries index and some hormonal and biochemical indicators. The immune system is not fully mature in young children, in addition to a lack of plasma cell generation, so the incomplete inflammatory process in children may explain the difference in results. The caries index also contains extracted and filled teeth, and these teeth have no biochemical effect (90,91).

If dental caries is not treated, it can lead to chronic inflammation triggered by a permanent bacterial infection in the teeth, and accordingly, the CRP level in the patient's serum increases. The host's immune response to bacterial infection and the release of inflammatory mediators from damaged dental tissues result in increased C-reactive protein (CRP) production (33).

Interleukin-21 is a cytokine with a wide variety of various effects on lymphoid and myeloid cell development and activity (43). IL-21 may play a role in tissue destruction. IL-21 can be used as a diagnostic biomarker in chronic periodontitis (48).

A study conducted by Lokhande RV et al. found an increase in serum IL-21 levels in patients with periodontitis. Elevated IL-21 levels may play a role in bone and periodontal tissue loss. This level has been used as a diagnostic indicator in chronic periodontitis. IL-21 is a pleiotropic cytokine and tends to affect immunity. IL-21 plays a role in the development of Th17 cells, which play an important role in the periodontitis disease process (48).

The immune response controls gingivitis at the beginning of the disease, but with the increase in bacterial activity, the intensity of the immune response increases, which leads to the destruction of periodontal tissues. The results of the study conducted by Gornowicz A showed an increase in the level of IL-6, TNF-α, and IL-8 in saliva in patients with dental caries. The increase in the level of TNF-α and IL-6 in saliva contributes to the demineralization of dental tissues affected by dental caries, which leads to the development of dental caries in patients (73).

We did not find many studies on the level of IL-21 in the serum of children with early childhood caries, but the results of our study were similar to studies on other cytokines in the serum of young children with ECC. According to Lima GQT et al. The level of cytokines increased in the serum of children with early childhood caries. Elevated serum levels of IL-6, TNF-α, and NGAL were associated with an increased number of teeth with caries. This indicates that chronic inflammation underlies early childhood caries (77).

In this study, we evaluated IL-21 levels in serum in children with ECC. The results showed higher IL-21 levels in the ECC group compared to the control group ($p < 0.001$). Cells in the dental odontoblast layer initiate immunological responses to dental caries through proinflammatory cytokine signaling (86). It would be biologically reasonable for children with ECC to have higher serum levels of proinflammatory cytokines because they are exposed to an unhealthy diet rich in added sugars and are at risk for obesity (77). The role of cytokines in the dental caries process is not well known. More information is needed on the molecular mechanisms of dental caries. Cytokines play a role in such a mechanism, but the details cannot be explained (73).

In our study, we did not notice a difference between males and females in the clinical measurements of the biochemical indicators that we examined. Our study was conducted on children with toothache, so we thought they had advanced caries in at least one of the teeth, and we could not exclude the presence of periodontal disorders. Most studies related to dental caries have studied biochemical indicators in saliva. Those researching serum biochemical indicators were interested in periodontitis. There has been very little research that examines serum biochemical markers and their relationships with early childhood caries. We need more studies that take into account the degree of tooth decay, the number of teeth affected, the degree of oral hygiene, and the absence of other oral diseases.

6. CONCLUSION

This study aimed to measure 8-OHdG, CRP, IL-21, and oxidative stress levels in the serum of children with early childhood caries.

In our study, we investigated whether there was a difference between the clinical measurement levels according to the patient and control groups. As a result of the research, it was found that the serum levels of 8-OHdG, TAS, TOS, OSI, IL-21, and CRP differed statistically in the patient and control groups. While 8-OHdG, TOS, OSI, IL-21, and CRP levels in the early childhood caries patient group were significantly higher than in the control group, TAS levels were significantly lower in the early childhood caries patient group.

The frequent use of biomarkers in dental treatments can lead to many results and interpretations in oral diseases. We suggest more research to figure out how the link between early childhood caries and serum biochemical markers works.

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