Systemic levels of inflammatory mediators in periodontitis

THESIS

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By

Dimitrios Malamis, MS

Graduate Program in Dentistry

The Ohio State University

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Master’s Examination Committee:

Dr. Dimitris N. Tatakis, Advisor

Dr. Binnaz Leblebicioglu

Dr. Timothy Eubank
Abstract

Background: Periodontitis has been associated with several medical conditions. For some of these medical conditions, periodontitis has been hypothesized to share important pathogenetic mechanisms. Recently, advances in biochemistry have led to the identification of novel inflammatory mediators implicated in some of the chronic medical conditions associated with periodontitis. However, there is a little to no evidence on the systemic levels of such biomarkers in periodontitis. Potential identification of these systemic inflammatory mediators in periodontitis would offer additional support to the potential periodontal-systemic disease association.

Methods: 33 systemically healthy persons, 16 periodontally healthy individuals and 17 chronic periodontitis patients, were recruited as part of an observational study. Peripheral blood samples were collected from each participant by venipuncture. Blood samples were collected twice from each participant, at two different time points 1-2 weeks apart. For 10 out of the 17 periodontitis patients who returned for reevaluation, following periodontal treatment, a third blood sample was collected. Samples were processed to prepare serum, aliquoted and stored at -80 °C. Aliquoted serum samples were analyzed for HMGB-1, S100A12, chemerin, BAFF, FGF-21 and ADMA levels using commercially available ELISA kits following the manufacturer’s instructions and performing the assay in duplicate wells.
Results: The results showed that the average serum levels of HMGB-1 and chemerin were statistically significantly higher in patients with periodontitis compared to healthy subjects. The levels of S100A12, BAFF, FGF-21 and ADMA were found to be elevated in the patient group compared to the control group but the differences did not reach statistical significance. Statistical analysis of the subgroup of patients that received nonsurgical periodontal treatment showed that the systemic levels of all inflammatory mediators, except for FGF-21, decreased following scaling and root planing. However the differences were not statistically significant.

Conclusion: Chronic periodontitis patients were found to have significantly higher serum levels of HMGB-1 and chemerin compared to periodontally healthy controls. The levels of the remaining 4 inflammatory mediators in the patient group were higher but not statistically significantly greater than the control group. The above findings support the existing evidence of an association between periodontitis and other systemic diseases/conditions and should form the impetus for more extensive research towards the identification of systemic biomarkers for periodontitis.
Dedication

Dedicated to Andreas, Katerina, Leonidas and Marialena
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VITA

2009 ...........................................................................................................DDS: University of Athens, Greece

2012 ...........................................................................................................MS: University of Aachen, Germany

Fields of study

Major Field: Dentistry
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Chapter 1

Introduction

Periodontitis and inflammation

Periodontitis is an inflammatory disease of the tooth supporting tissues. It is caused by the inflammatory response of the gingival tissues and the subjacent bone to the presence of dental plaque and leads to nonreversible loss of tooth support. "Dental plaque is a filmy deposit on the surface of a tooth consisting of a mixture of mucus, bacteria and food" according to Collin's dictionary. Although originally it was believed that the etiology of periodontal disease could be attributed to plaque mass (non-specific plaque hypothesis), later studies supported the notion that specific bacteria are responsible for the tissue destruction. Most recently, focus has slightly shifted from specific pathogens to the composition and activity of the biofilm present in the periodontal niche. Biofilms are defined as "oriented aggregations of microorganisms attached to each other or to a surface and enclosed in extracellular polymeric substance produced by themselves". There are various methods of interaction between the diverse microbial species of the biofilm including physical contact, small signal molecules and metabolic and genetic material exchanges. Microbial communities in periodontal disease are more active in the metabolism of certain carbohydrates compared to microbial communities present in
periodontal health. Such results indicate microbial functional diversity between periodontally healthy and periodontally diseased tissues. In fact the above distinction based on ecology and function is not only true about bacteria but also about viruses, which constitute part of the biofilm.

The manner in which bacteria interact with the host is primarily through their virulence factors. Bacterial components and products such as the LPS (lipopolysaccharide) of gram negative bacteria, the gingipains and hemagglutinins of P.gingivalis and the leukotoxin and cytolethal distending toxin of A. actinomycetemcomitans interact with host cells and induce an innate and humoral immune response. Although there is great inter-individual variation in the immune response to the bacterial challenge, this response is mainly responsible for the loss of periodontal tissues. The first event that occurs as part of this response is vessel dilation, release of pro-inflammatory factors such as PGE-2 and IL-1β and expression of adhesion molecules on the endothelium of the neighboring vessels. Neutrophils are attracted to the site via chemotaxis mediated by QS (quorum sensing) molecules of the N-acyl homoserine lactone family as well as specific bacterial components. When they reach the biofilm area, they initiate processes such as phagocytosis, degranulation, formation of neutrophil extracellular traps (NETosis), and release of reactive oxygen species (ROS) in an effort to contain and eliminate the bacteria. As another line of defense, macrophages also migrate in the area of inflammation where they participate in phagocytosis and opsonization. These cells secrete large amounts of TNF-α and IL-1β, which are pro-inflammatory and play an important role in bone resorption through the activation of proteolytic molecules known
as MMPs (matrix metalloproteinases). With time the inflammatory infiltrate is dominated by B-lymphocytes and plasma cells. In 1976 Page and Schroder attempted to describe the sequence of these events and proposed 4 disease stages based on histopathological findings in inflamed gingiva.

1. **Initial lesion**: Vasculitis of vessels subjacent to junctional epithelium, increased migration of leukocytes into junctional epithelium, extravascular presence of serum proteins, especially fibrin, alteration of the most coronal portion of junctional epithelium and loss of perivascular collagen.

2. **Early lesion**: Accentuation of features of initial lesion, accumulation of lymphocytes, 70% loss of perivascular collagen, cytopathic alterations of fibroblasts

3. **Established lesion**: Predominance of plasma cells, proliferation and apical migration of junctional epithelium

4. **Advanced lesion**: Continued loss of collagen, extension of lesion into periodontal ligament and bone and formation of periodontal pocket.

Clinically, the above processes manifest themselves as inflammation, which leads to tissue degradation. With a growing understanding of the pathogenesis of periodontal disease it has become evident that a linear model involving a bilateral interaction between bacteria, host response and clinical signs and symptoms is no longer applicable to it. The degree and rate of progression of tissue destruction are influenced by other factors such as genetics, smoking status, stress and systemic health conditions. Furthermore, these factors exert their influence on multiple cellular levels such as gene expression,
molecular signaling, metabolism, differentiation, phenotypic expression and intercellular interaction before they exhibit their influence as clinical signs of disease. These series of events are orchestrated and controlled through the release of arachidonic metabolites, complement components and cytokines. The etymology of the latter is Greek and derives from the word cyto- meaning “cell” and kinos- meaning movement; and it is used to describe molecules used for cell signaling in the modulation of immune response.

If left untreated, periodontitis can lead to tooth loss, consequently impairing function, esthetics and quality of life. In the U.S. the prevalence of periodontitis among people ≥30 years of age was calculated to be 47.2% in a recent report based on the NHANES 2009-2010 data. In fact, among people ≥65 years old the prevalence was 70.1%. The above recent data were met with surprise since in the past the prevalence of periodontitis was underestimated significantly, in terms of both prevalence as well as severity. The reason for the prior underestimation was the data collection protocol used (partial mouth probing in selected sites). The recently reported increased prevalence of periodontitis has additional significance given the reported associations between oral disease (specifically periodontal disease) and systemic disease (diseases such as diabetes, cardiovascular disease, etc.) The strong association of periodontitis with several systemic diseases has been attributed to a number of factors, including systemic distribution of periodontal pathogens and systemic leakage of local inflammatory mediators. There is evidence for the systemic distribution of both periodontal pathogens and local inflammatory mediators. The local inflammatory mediators can elicit systemic effects, and thus
contribute to systemic diseases, through direct effects on tissues, such as vessel endothelium, and through indirect effects via stimulation of the liver, which in turn releases molecules (e.g., C-reactive protein) that have direct, tissue-specific effects.\textsuperscript{18}

**Evidence for association between periodontitis and systemic diseases**

One of the most extensively investigated systemic diseases in periodontal literature is diabetes type 2.\textsuperscript{19} In a review paper from 2012 by Javed it was reported that some of the existing studies indicate that IL-6, IL-17 and IL-23 are elevated in the gingival crevicular fluid of patients with periodontitis and diabetes type 2 compared to systemically healthy periodontitis patients.\textsuperscript{20} Taylor also reported that most of the studies show elevated levels of IL1-β and IL-6 in diabetic patients with periodontal disease compared to systemically healthy periodontal patients. Since the microbiological profile of these two groups of periodontal patients appears to be similar, a different mechanism of association between the two diseases has been proposed.\textsuperscript{21} Uncontrolled diabetes seems to exert its influence on the periodontal tissues via a number of different pathways.\textsuperscript{21} Firstly, hyperglycemia has a direct effect on cells by increasing their cellular stress. Secondly, the reactive oxygen species being produced upregulate the production of pro-inflammatory cytokines. Finally, in uncontrolled diabetic patients, there is increased production of Advanced Glycation End-products (AGEs). The latter, when bound to the corresponding receptor (RAGE), induce phenotypic and functional changes on cells and enhance inflammation and oxidative stress. Furthermore, they seem to have an influence in bone homeostasis by increasing the RANKL/OPG ratio, which promotes osteoclast activation and bone
resorption. It is not only diabetes that influences periodontitis. There is ample evidence supporting a reverse association as well. Inflammation in the periodontal tissues and increases in TNF-α and IL-6 levels in the systemic circulation increases insulin resistance of the host, which in turns results in poorer diabetic control.

Another group of systemic diseases, which has been associated with periodontitis are cardiovascular diseases. In his study Loos in 2000 showed that the levels of IL-6 and C-reactive protein (CRP), which have already been identified as risk factors for cardiovascular disease, are statistically significantly elevated in patients with periodontal disease versus controls. CRP seems to be related to the release of reactant proteins, such as fibrinogen, which cause thrombosis. IL-6 seems to be promoting vascular thrombosis through the release of leukocytes and platelets into the systemic circulation and through alteration of hepatic or endothelial synthesis and release of plasma proteins. Other potential mechanisms of association that have been proposed involve a number of different molecules, which are typically elevated due to periodontal disease. Such molecules include IL-1, TNF-α, IL-12, ICAM-1, VCAM-1, Heat shock proteins (HSPs) and fibrinogen, which have been implicated in the formation of atheromatic plaques on vessel walls. Since periodontal treatment reduces inflammation, it leads to a decrease in the systemic levels of many of the above atherosclerotic disease biomarkers and improves endothelial function.

Autoimmune diseases have also been associated with periodontal disease. A significant number of studies has focused on rheumatoid arthritis (RA). De Pablo in 2008 showed that patients with rheumatoid arthritis miss more teeth than patients without
rheumatoid arthritis (20 vs 16). In her study it was also shown that rheumatoid arthritis patients are more likely to become edentulous and have periodontitis (O.R. of 2.27 and 1.82 respectively) compared to non rheumatoid arthritis individuals. In his review paper Javed, reported that there are only a few studies have investigated the GCF levels of pro and anti-inflammatory cytokines in patients with and without rheumatoid arthritis. In some of these studies IL-1β, TNF-α, MMP-8 and MMP-13 were found elevated in the rheumatoid arthritis group. The mechanism of association which has been suggested involves the release of pro-inflammatory cytokines in the systemic circulation and the activation of Th-17 cells which mediate joint inflammation and bone destruction. Although there is a paucity of well-controlled human studies, animal models have shown that periodontal pathogens play a distinct role in the progression and exacerbation of experimental arthritis.

**Significance of biomarker research in periodontology**

The most common diagnostic tools for periodontitis, used since the early 1960s and still implemented today, involve the measurement of probing depths, attachment loss, evaluation of clinical signs of inflammation (e.g., bleeding, purulent exudate, erythema, edema) and radiographic evaluation of the alveolar bone. Careful consideration of the clinical findings guides the clinician to establish a diagnosis based on an accepted classification system of periodontal diseases and conditions. More recently, molecular determinants have been used to try to establish a more biologically-based diagnostic approach and to explain the various associations between periodontitis and systemic
diseases.

As previously stated researchers have been trying to investigate the levels of inflammatory mediators in patients with periodontitis and healthy individuals in an attempt to develop new diagnostic tools that enable them to determine not only the presence of periodontal disease but also to measure its activity in terms of the degree of inflammation and/or tissue destruction. However, almost none of the studies available in the literature have looked into the systemic levels of a novel group of cytokines termed “alarmins” in periodontal disease. The purpose of this study was to examine the systemic levels of alarmins and novel biomarkers implicated in periodontitis-associated systemic diseases in patients with periodontitis and healthy controls.

1. Alarmins: An overview

Alarmins are a subgroup of a larger set of damage-associated molecules. According to Garg et al “Damage-associated Molecular patterns (DAMPs) are molecules that are secreted, released or surface exposed by dying, stressed or injured cells. DAMPs can function as either adjuvant or danger signals for the immune system”. The term alarmins was first proposed by Joost Oppenheim to differentiate the endogenous molecules that signal tissue and cell damage. They were defined as “endogenous mediators that can simultaneously induce the chemotactic migration and activation of antigen-presenting cells (APCs) and consequently promote the induction of immune responses.” This dual effect of alarmins distinguishes them from other pro-
inflammatory cytokines whose role is limited to the activation of APCs and which lack chemoattracting properties.\textsuperscript{34}

More specifically, the following are the characteristics that alarmins possess:

1) Rapid release following cellular necrosis (but not after regular apoptosis).
2) Release from immune system cells that are not dying by specialized secretion systems or by the endoplasmic reticulum - Golgi secretion pathway.
3) Recruitment and activation of receptor-expressing cells of the innate immune system.
4) Capacity to reestablish homeostasis by promoting reconstruction of the tissue lost due to the inflammation process.\textsuperscript{35}

Alarmins are considered to be the link between tissue injury and inflammatory reaction. They function together with another group of DAMPs named pathogen associated molecular patterns (PAMPs) in a synergistic manner to augment the inflammatory response.\textsuperscript{36} PAMPs are a group of microbial molecules that alert the host to the pathogenic intruder.\textsuperscript{35}

In general, circulating levels of pro-inflammatory mediators depend on the extent of the periodontal lesion. According to Hujoel et al., the total surface area of the periodontal ligament exhibiting inflammation in periodontitis ranges from 1-44 cm\textsuperscript{2}.\textsuperscript{37} This means that in severe cases the total ulcerated area can be of significant extent. The inflamed subgingival epithelium comprises the entry port for bacteria and antigens to the systemic circulation.\textsuperscript{37} As a reaction to this bacteremia, blood cells and tissue cells from areas where the bacteria and the antigens migrate into, respond with the production of inflammatory mediators. Additionally, the mediators found in the gingival crevicular
fluid are discarded in the systemic circulation further increasing their concentration in it.\textsuperscript{18}

2. Specific alarmins: HMGB-1 and S100A12

- **HMGB-1**

HMGB-1 is a nuclear protein that belongs to the high mobility group (HMG) superfamily of nuclear proteins first discovered in 1973. HMGB-1 can be found in the nucleus of eukaryotic cells with usual concentration of $10^6$ molecules per cell. Its main functions are associated with DNA architecture and regulation of transcription. Structurally it is comprised of two “boxes”, A and B, each one made of approximately 80 aminoacids. Most studies have attributed the pro-inflammatory properties of HMGB-1 to its B-box domain.\textsuperscript{38} HMGB-1 is released passively during cellular necrosis and it is also actively released in the presence of other cytokines, cellular stress or PAMPs by immune cells such as macrophages, monocytes and dendritic cells.\textsuperscript{39} After being released it binds to TLR or RAGE, inducing a series of effects. It stimulates monocyte adhesion and cytokine release, it enhances neutrophil migration, adhesion and reactive oxygen species production, and it induces endothelial cell release of TNF-a, IL-8, MCP-1, ICAM-1, and VCAM-1, thus amplifying the inflammatory state.\textsuperscript{40}

HMGB-1’s involvement in the development of inflammation has been extensively investigated in conditions such as autoimmune diseases, cancer, acute trauma, ischemia
and type-2 diabetes, among others.\textsuperscript{41-45} In the field of periodontology, a recent study has examined the concentration of HMGB-1 in the crevicular fluid around teeth and implants and reported increased levels of HMGB-1 in the fluid of diseased periodontal or periimplant tissues as opposed to healthy gingiva.\textsuperscript{46} No other similar studies or studies investigating HMGB-1 systemic levels in periodontal patients have been reported, to the best of our knowledge. The potential significance of such studies is highlighted by new hypotheses suggesting that HMGB-1 could serve as the link between periodontitis and systemic conditions such as type-2 diabetes.\textsuperscript{43}

- **S100A12**

S100 proteins comprise one of the subgroups of the EF-hand calcium-binding proteins. Their main role is to modulate the action and/or distribution of specific proteins upon calcium-dependent activation.\textsuperscript{47} S100A12 along with S100A8 and S100A9 form a subgroup named calgranulins.\textsuperscript{48} S100A12 was first described by Guignard et al. as a neutrophil granulocyte cytosolic protein.\textsuperscript{49} The most important target of S100A12 is RAGE. The S100A12 gene is approximately 4.1 kbp long and has 3 exons and 2 introns. The binding of S100A12 to the extracellular domain of RAGE activates MAP-kinase, NFκB, secretion of pro-inflammatory cytokines (TNF-α, IL-1β) and the expression of VCAM-1 and ICAM-1. Consequently, S100A12 exerts its pro-inflammatory effect on lymphocytes, endothelial cells, neutrophils and mononuclear phagocytes. It has been shown that S100A12 gene expression is mainly limited to neutrophil granulocytes and that monocytes are able to
express S100A12 to a small extent.\textsuperscript{49}

Expression of S100A12 characterizes many systemic diseases such as Chron’s disease, acute vasculitis syndromes, juvenile idiopathic arthritis, rheumatoid and psoriatic arthritis, respiratory distress syndrome, atherosclerotic related inflammation and type-2 diabetes, among others.\textsuperscript{50} For that reason, serum S100A12 measurements have been suggested to be of diagnostic capacity. In fact, the diagnostic value of S100A12 appears to be superior to many other traditional parameters of inflammation since it correlates to local inflammatory processes, which involve activation of granulocytes and monocytes. The systemic levels of S100A12 in periodontal disease have only been reported in one study.\textsuperscript{51}

3. Other systemic biomarkers: Chemerin, BAFF, FGF-21 and ADMA

- **Chemerin**

Chemerin was first described in 2003 by Wittamer as a precursor molecule to a potent agonist of the ChemR23 receptor.\textsuperscript{52} The chemerin receptor has been found in antigen presenting cells (APCs) and specifically in macrophages and immature dendritic cells (DCs). Chemerin was first isolated from the inflammatory fluids of patients with rheumatoid arthritis, ovarian cancer and liver cancer.\textsuperscript{52} Structurally, it is made of a cystatin fold similar to extracellular proteins including cystatins type2, cathelicidins and kininogen. Many functions have been attributed to chemerin. It has been found to be a very potent chemoattractant of leukocyte cells such as APCs and natural killer cells. Most data indicate that it can have a pro or anti-inflammatory effect depending on the disease
model.\textsuperscript{53} In conditions such as inflammatory bowel disease\textsuperscript{54}, rheumatoid arthritis\textsuperscript{55} and diabetes type 2 induced retinopathy\textsuperscript{56} it seems to have a pro-inflammatory function. Due to the similarities in its structure to cathelicidins, a broad-spectrum antimicrobial role of chemerin in human keratinocytes that form the dermis barrier has also been proposed.\textsuperscript{57} Chemerin, considered an adipokine that regulates both adipocyte function and glucose metabolism in liver and skeletal muscle tissues, shows a positive correlation with various aspects of the metabolic syndrome and appears to have a dual role in inflammation and metabolism; this might be a link between chronic inflammation, such as periodontal disease, and obesity (and obesity-related disorders such as type 2 diabetes and cardiovascular disease).\textsuperscript{53, 58, 59} Although systemic levels of chemerin in periodontal disease have not yet been reported, the results of recent studies on levels of other adipokines in periodontal disease have been mixed.\textsuperscript{60-64}

- **BAFF**

B-cell-activating factor (BAFF), also called B-lymphocyte stimulator (Blys), or TNF and apoptosis leukocyte-expressed ligand 1 (TALL-1), is a homotrimer belonging to the TNF superfamily and was first described in 1999.\textsuperscript{65, 66} BAFF is being produced as a 285-aminoacid type II transmembrane protein and can be cleaved by furin protease resulting in a soluble form which is important for B-cell maturation. BAFF can form homotrimers or even heterotrimers with a similar protein APRIL (30\% homology) and it is expressed in a variety of cell types including monocytes, lymphocytes as well as cells of hematopoietic lineage.\textsuperscript{67, 68} It's interaction with cells is mediated through 3 receptors;
TACI I (transmembrane activator and calcium modulator and cyclophilin ligand interactor), BCMA (B cell maturation antigen) and BAFFR receptor (also known as BR3).\textsuperscript{69} BAFF has been identified as an important growth factor for plasma cells in multiple myeloma and it has been implicated in the pathogenesis and/or progression of conditions such as autoimmune hemolytic anemia, non-hodgkin lymphoma, chronic obstructive pulmonary disease and systemic lupus erythematosus.\textsuperscript{67, 70-74} More importantly for the context of periodontal disease, BAFF has been shown to be produced by neutrophils and to be involved in T-cell activation, proliferation and differentiation\textsuperscript{75, 76}, is elevated in the serum of patients with rheumatoid arthritis (RA) and has been implicated in tissue inflammation.\textsuperscript{77-80} Furthermore, one study performed on patients with Sjögren's syndrome has shown a correlation between the salivary levels of BAFF and the severity of periodontal disease\textsuperscript{81} and two recent studies have shown elevated levels of BAFF in the gingival crevicular fluid and/or serum of periodontitis patients.\textsuperscript{82, 83}

\begin{itemize}
\item \textbf{FGF-21}
\end{itemize}

Fibroblast growth factors (FGFs) are proteins comprised of approximately 150-300 amino acids and they have been linked to physiological processes such as development, transformation and angiogenesis. FGFs may have endocrine, intracrine or paracrine function.\textsuperscript{84} FGF21 shares sequence identities with FGF15/FGF19 and FGF23 and they all exhibit an endocrine mechanism of action mediated mainly by FGF receptors.\textsuperscript{84, 85} FGF21 is expressed in a number of tissues such as liver, brown and white adipose tissue, and pancreas and its functions are mainly mediated through bKlotho/FGFR1 receptor.\textsuperscript{86, 87} It
appears to have metabolic but not proliferative properties and it is mainly involved in fat and carbohydrate metabolism.\textsuperscript{85,86} It may serve as hepatokine, myokine and adipokine.\textsuperscript{84} Its role in the development and progression of diabetes type 2 has been extensively investigated in the literature and its levels positively correlate with the severity of glycaemia and the levels of insulin resistance.\textsuperscript{88} Some studies have found an association between the levels of FGF21 in diabetic patients with subclinical atherosclerosis and diabetic retinopathy.\textsuperscript{89,90} Additionally, serum levels of FGF21 have been positively correlated with atrial fibrillation and carotid atherosclerosis.\textsuperscript{91,92} Most of the recent studies indicate that FGF21 plays a major role in metabolic homeostasis by increasing insulin sensitivity and reducing dyslipidemia.\textsuperscript{88,93} Additionally, it has been shown to have a protective effect in the heart by preventing the formation of ROS in cardiac cells and by preventing cardiac hypertrophy.\textsuperscript{94,95} These findings indicate that increased levels of FGF21 in pathological conditions such as adverse lipid profiles in coronary heart disease may be interpreted as a compensatory mechanism or as a sign of tissue resistance to FGF21.\textsuperscript{84} The levels of FGF21 in periodontal disease have not yet been investigated.

- ADMA

Asymmetric dimethylarginine is a naturally modified amino acid found in the systemic circulation, first identified in 1987.\textsuperscript{96,97} Studies have shown that it is metabolized by NG, NG\textsuperscript{-}dimethylarginine dimethylaminohydrolase (DDAH) to citrulline and dimethylamine.\textsuperscript{96} It is released from proteins usually located in the nucleus, which have been post-translationally modified and it is a potent competitive inhibitor of NOS (Nitric
Oxide Sunthase).\textsuperscript{98} These proteins are mostly associated with RNA processing and transcriptional control.\textsuperscript{99} ADMA levels have been suggested as a systemic biomarker for a number of pathological conditions/diseases. Regarding cardiovascular diseases, circulating ADMA levels have been positively correlated with carotid intima-media thickness.\textsuperscript{100} A recent systematic review and meta-analysis of 22 prospective studies concluded that systemic ADMA levels appear to be associated with cardiovascular disease outcomes.\textsuperscript{97} In patients with diabetes mellitus, increased plasma concentration of ADMA seemed to have a predictive value of cardiovascular complications within a time frame of 5 years.\textsuperscript{101} Except for its involvement in diabetes and CVD, ADMA has also been associated with pathological processes leading to progression of chronic kidney disease (CKD).\textsuperscript{102} According to another review paper, ADMA may be implicated in the pathological mechanisms of cardiovascular events in a number of metabolic and autoimmune disorders including diabetes mellitus, rheumatoid arthritis, systemic lupus erythematosus and systemic sclerosis.\textsuperscript{98} Although there are no studies investigating local (gingival crevicular fluid) or systemic (serum/plasma) levels of either ADMA or SDMA in chronic or aggressive periodontitis, two studies from the same research group have reported elevated serum ADMA levels in patients with endodontic lesions and apical periodontitis.\textsuperscript{103, 104}
**Working Hypothesis**

Given the association of periodontal disease with certain systemic diseases, and the discovery of new biomarkers for those diseases, we hypothesize that:

1. In patients with periodontal disease the systemic levels of the pro-inflammatory mediators HMGB-1, S100A12, chemerin, BAFF, FGF-21 and ADMA are elevated compared to periodontally healthy individuals.

2. The systemic levels of these pro-inflammatory mediators decrease following non-surgical periodontal treatment.

**Specific Aims**

1. a. To investigate the systemic levels of the pro-inflammatory mediators; HMGB-1, S100A12, chemerin, BAFF, FGF-21 and ADMA in patients with periodontitis and in periodontally healthy individuals.

   b. To monitor the levels of the above inflammatory mediators at two time points.

2. To determine whether there are differences in the levels of the above mediators among periodontitis patients before and after non-surgical periodontal treatment.
Chapter 2

Materials and Methods

This observational study included two groups of participants that were asked to complete study questionnaires and provide blood samples at different time points. The blood draws and questionnaires were the only study procedures. The included participant groups were:

1) 17 patients diagnosed with generalized chronic periodontitis seeking periodontal treatment at the OSU College of Dentistry. Patients had to have received the disease diagnosis and agreed to a non-surgical periodontal treatment plan prior to be eligible for this study.

2) 16 periodontally healthy control subjects presenting to the OSU College of Dentistry for any treatment need other than periodontitis.

Exclusion criteria for all patient groups: patients <21 years old and >70 years old (due to the increased likelihood of having more significant medical history), patients with drug or alcohol abuse, rheumatoid arthritis, diabetes type I or II, use of antibiotics within the last 3 months, periodontal treatment (non-surgical or surgical) within the last 6 months, systemic use of NSAIDS or other anti-inflammatory drugs, acute coronary syndrome,
other autoimmune or systemic diseases that may alter the course of periodontal treatment, pregnant or lactating women, patients categorized as ASA 3 or higher. Participants for the periodontally diseased and periodontally healthy group were identified based on the findings of their scheduled periodontal examination. This included calculation of the percentage of surfaces (6 sites/tooth) exhibiting bleeding on slight probing and the percentage of surfaces (6 sites/tooth) exhibiting plaque, full mouth probing and charting (using a calibrated probe) and calculation of the probing depth, the gingival margin position (gingival recession) and the attachment level per surface of the tooth (6 sites/tooth). Additionally, full mouth series of periapical radiographs using the long cone technique were taken and the radiographic bone loss per tooth was estimated (mesial and distal). All the above procedures were done as part of the initial periodontal examination of every single patient presenting in the graduate periodontology clinic.

1. **Chronic Periodontitis (CP) group**

   a. Patient sample

Patients with chronic periodontitis (according to the criteria set by Armitage)\(^{31}\) in order to qualify for the study had to have: at least 14 periodontally evaluable natural teeth present (excluding third molars); \(\geq 4\) teeth with \(\geq 1\) sites with periodontal probing depth (PPD) \(\geq 5\) mm and clinical attachment level (CAL) \(\geq 3\) mm at same site, \(^{105, 106}\) radiographic bone loss \(\geq 20\%\) at same site; baseline whole mouth bleeding on probing (BOP) \(> 20\%\) of sites; no mechanical/antibiotic/anti-inflammatory/surgical periodontal therapy in the last 6 months; no other oral diseases (e.g. oral/mucosal lesions and oral
infections); no immediate need for extractions and no recent (<2 months) tooth extractions.

b. Study design

Subjects of this group received a first blood draw (baseline cytokine levels) after agreeing to participate in the study, signing the informed consent and completing a questionnaire. After 1-2 weeks a second blood draw was performed (2nd baseline cytokine levels). On the same day, and prior to the blood draw, patients completed a questionnaire (previously developed and used in similar studies by the principal investigator) to determine whether any changes in their medical or oral condition or prescriptions had occurred within that period of time (continued eligibility). After the second blood draw, patients started to receive the planned treatment, i.e. routine, non-surgical periodontal therapy, which consisted of scaling and root planing (“deep cleaning”), to be performed within 4 weeks. The treatment itself was not a study procedure. A third and final blood sample was collected 8-12 weeks following the day of scaling and root planing completion; at that time patients were asked to complete a final questionnaire to determine possible changes in medical or oral condition or prescriptions that occurred during this 8-12 week period. This third appointment was the study endpoint for the periodontitis patients.

Therefore, the timetable of the various study time points (appointments) and study procedures for the chronic periodontitis (CP) group went as follows:
<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><strong>$T_1=\text{Time 0=Baseline 1}$</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Review, verification of eligibility, and collection of signed informed consent, HIPAA forms, Questionnaire</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• 1$^{\text{st}}$ Blood Draw</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td><strong>$T_2=1-2 \text{ weeks after } T_1=\text{Baseline 2}$</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Questionnaire, verification of continued eligibility</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• 2$^{\text{nd}}$ Blood Draw</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td><strong>$T_3=11-18 \text{ weeks } = \text{Endpoint (8-12 weeks after completion of periodontal non-surgical therapy)}$</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Questionnaire, verification of continued eligibility</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• 3$^{\text{rd}}$ Blood Draw</td>
<td></td>
</tr>
</tbody>
</table>

2. **Periodontally healthy (PH) group**

a. Population sample

Individuals determined to be periodontally healthy had to have: at least 14 periodontally evaluable natural teeth present (excluding third molars); full mouth BOP ≤20% sites; full mouth plaque score ≤30% sites; all teeth (excluding third molars) having probing depths ≤4mm at all sites; no interproximal surfaces with attachment loss >2mm; and no radiographic bone loss greater than 10%; no mechanical/antibiotic/anti-
inflammatory/surgical periodontal therapy in the last 6 months; no other oral diseases (e.g. oral/mucosal lesions and oral infections); no immediate need for extractions and no recent (<2 months) tooth extractions.

b. Study design

Subjects of this group received a first blood draw (baseline cytokine levels) after agreeing to participate in the study, signing the informed consent and completing a questionnaire. After 1-2 weeks a second blood draw will be performed (2nd baseline cytokine levels). On the same day, and prior to the blood draw, patients completed a questionnaire to determine whether any changes in their medical or oral condition or prescriptions have occurred within that period of time (continued eligibility). After the second blood draw, patients of this group were terminated from the study. They then continued to receive any dental/periodontal treatment that had been planned prior to study entry.

Therefore, the timetable of the various study time points (appointments) and study procedures for the periodontally healthy (PH) group went as follows:
<table>
<thead>
<tr>
<th>1</th>
<th>( T_1 = \text{Time 0 = Baseline 1} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Review, verification of eligibility, and collection of signed informed consent, HIPAA forms, Questionnaire</td>
<td></td>
</tr>
<tr>
<td>• 1(^{st}) Blood Draw</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>2</th>
<th>( T_2 = 1-2 \text{ weeks after } T_1 = \text{Baseline 2} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Questionnaire, verification of continued eligibility</td>
<td></td>
</tr>
<tr>
<td>• 2(^{nd}) Blood Draw</td>
<td></td>
</tr>
</tbody>
</table>

**Blood collection and assay**

Blood collection was performed via venipuncture on the right or left antecubital fossa. Ten milliliters of peripheral blood were collected using a 23-gauge needle, with 5 ml collected in a serum preparation tube (tube with clot activator and gel) and 5 ml collected in a tube with EDTA as anticoagulant (plasma preparation). The samples were transferred to the laboratory within 15 minutes following collection and were immediately centrifuged to prepare serum or plasma, as indicated. The prepared serum/plasma samples were aliquoted and stored frozen at -80°C until further analysis by antibody-based assays to determine systemic concentration of the inflammatory mediators of interest. The cellular blood components remaining after serum/plasma preparation were discarded.

Aliquoted serum and plasma samples were assayed for HMGB-1, S100A12, chemerin,
BAFF, FGF-21 and ADMA levels using commercially available enzyme-linked immunosorbent assay (ELISA) kits, according to manufacturer’s instructions. When needed, dilutions were performed and included in the final result calculations following the manufacturer's recommendations.

Statistical analyses

The primary outcome of this study was the difference in systemic levels of specific biomarkers between periodontitis patients and controls (cross-sectional assessment). The sample size of the two groups (periodontitis and healthy controls) was based on previous studies of similar design. Specifically, a study with 32 participants (16 per group) has 80% power to detect a 20% difference in mean levels between two independent groups (equal variance; sigma=0.2), assuming an alpha of 0.05. All parameters that were measured in the study are presented using descriptive statistics (means and standard deviations). Depending on data normality, intra-group differences at various time points were analyzed using paired t-test or Wilcoxon Signed Rank Test. Depending on data normality, inter-group differences were analyzed using t-test or Mann-Whitney Test. The level of significance was set at a=0.05.
Chapter 3

Results

Demographics

In total 16 healthy controls and 17 chronic periodontitis patients were recruited in the study. Ten out of the 17 patients completed the non-surgical periodontal treatment and returned for a reevaluation. The mean age of periodontally healthy subjects was 48 years and the mean age of periodontal patients was 46.9 years. The healthy group consisted of 9 men and 7 women; 12 were of Caucasian origin, 2 of Hispanic, 1 of African American and 1 of Asian. The periodontitis group consisted of 10 men and 7; 8 were of Caucasian, 5 of African American, 2 of Hispanic and 2 of Asian origin. In terms of smoking habits, one patient in the healthy group was a heavy smoker (arbitrarily set at ≥5 pack years tobacco exposure) and one was a smokeless tobacco user. In the chronic periodontitis group there were 4 participants who were determined to be light smokers (set at <5 pack years) and 3 who were classified as heavy smokers (as defined above). Regarding the severity of periodontal disease, the majority of the patients were diagnosed with generalized moderate localized severe chronic periodontitis.
Detailed demographics of the periodontally healthy and the periodontitis group can be seen on the following tables (Tables 1 and 2).
### Table 1: Demographic data of the periodontally healthy group

<table>
<thead>
<tr>
<th>SUBJECT</th>
<th>SEX</th>
<th>RACE</th>
<th>AGE</th>
<th>SMOKING</th>
<th>MEDICATION</th>
<th>MOUTH RinSE</th>
<th>SUPPLEMENTS</th>
<th>EXERCISE</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMH001</td>
<td>M</td>
<td>Caucasian</td>
<td>30</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>DMH002</td>
<td>F</td>
<td>AA</td>
<td>22</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>DMH003</td>
<td>M</td>
<td>Hispanic</td>
<td>43</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>Yes (Omega 3, multivitamin)</td>
<td>No</td>
</tr>
<tr>
<td>DMH004</td>
<td>M</td>
<td>Caucasian</td>
<td>65</td>
<td>No</td>
<td>Yes (Losartan, Pravastatin)</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
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<td>Caucasian</td>
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<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
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<td>F</td>
<td>Caucasian</td>
<td>40</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>DMH007</td>
<td>M</td>
<td>Caucasian</td>
<td>66</td>
<td>Yes (76 packyears)</td>
<td>Yes (Crestor, Flomox)</td>
<td>Yes</td>
<td>Yes (Vitamin D)</td>
<td>Yes</td>
</tr>
<tr>
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<td>Hispanic</td>
<td>45</td>
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<td>No</td>
<td>Yes</td>
<td>Yes (GAGs, Calcium, B12)</td>
<td>Yes</td>
</tr>
<tr>
<td>DMH009</td>
<td>F</td>
<td>Caucasian</td>
<td>57</td>
<td>No</td>
<td>Yes (Cyndera, Lanacal, Hyclide, Clozarxpan)</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>DMH010</td>
<td>M</td>
<td>Caucasian</td>
<td>64</td>
<td>No</td>
<td>Yes (Simvastatin, Allopornol, Synthroid)</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
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<td>DMH011</td>
<td>M</td>
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<td>49</td>
<td>No but Smokerless</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
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<td>Caucasian</td>
<td>49</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Yes (Vit. Calc, Iron)</td>
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</tr>
<tr>
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<td>M</td>
<td>Asian</td>
<td>34</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
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<td>No</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
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<tr>
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<td>61</td>
<td>No</td>
<td>Yes (Synthroid)</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>DMH017</td>
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<td>Caucasian</td>
<td>37</td>
<td>No</td>
<td>Yes (Losartan, Tramona, VitD3)</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>SUBJECT</td>
<td>SEX</td>
<td>RACE</td>
<td>AGE</td>
<td>SMOKING</td>
<td>MEDICATION</td>
<td>MOUTH RINSE</td>
<td>SUPPLEMENTS</td>
<td>EXERCISE</td>
</tr>
<tr>
<td>-----------</td>
<td>-----</td>
<td>------</td>
<td>-----</td>
<td>---------</td>
<td>----------------------------------------</td>
<td>-------------</td>
<td>-------------</td>
<td>----------</td>
</tr>
<tr>
<td>DMC002</td>
<td>F</td>
<td>AA</td>
<td>48</td>
<td>No</td>
<td>Yes (Losartan, Metoprolol, Moxapazine)</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>DMC003</td>
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<td>AA</td>
<td>38</td>
<td>No</td>
<td>Yes (Losartan, Metoprolol, Moxapazine)</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>DMC004</td>
<td>F</td>
<td>Caucasian</td>
<td>57</td>
<td>No</td>
<td>Yes (Methadone, Hydrochloride)</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>DMC005</td>
<td>M</td>
<td>Asian</td>
<td>44</td>
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<td>No</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>DMC006</td>
<td>M</td>
<td>Caucasian</td>
<td>59</td>
<td>Yes (1 packyears)</td>
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<td>Yes</td>
<td>No</td>
<td>No</td>
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<td>AA</td>
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<td>Yes (4 packyears)</td>
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<td>No</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>DMC009</td>
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<td>Caucasian</td>
<td>44</td>
<td>No</td>
<td>Yes (Risperidone)</td>
<td>Yes</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>DMC010</td>
<td>F</td>
<td>AA</td>
<td>48</td>
<td>Yes (3 packyears)</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>DMC012</td>
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<td>Hispanic</td>
<td>42</td>
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<td>No</td>
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<td></td>
</tr>
<tr>
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<td>Yes (Asacol)</td>
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<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>DMC014</td>
<td>F</td>
<td>Hispanic</td>
<td>38</td>
<td>No</td>
<td>Yes (Doxepin)</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>DMC015</td>
<td>M</td>
<td>AA</td>
<td>59</td>
<td>Yes (3 packyears)</td>
<td>Yes (Losartan, Omeprazole)</td>
<td>Yes</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>DMC016</td>
<td>M</td>
<td>Caucasian</td>
<td>46</td>
<td>Yes (8 packyears)</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>DMC017</td>
<td>F</td>
<td>Caucasian</td>
<td>45</td>
<td>Yes (20 packyears)</td>
<td>No</td>
<td>No</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DMC018</td>
<td>F</td>
<td>Hispanic</td>
<td>38</td>
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<td>No</td>
<td>Yes</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>DMC019</td>
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<td>Caucasian</td>
<td>48</td>
<td>Yes (20 packyears)</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>DMC020</td>
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<td>Asian</td>
<td>28</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>

Table 2: Demographic data of the periodontitis group
General information on cytokine analysis

In total we measured the systemic levels of 6 cytokines in the serum/plasma of the two groups of participants. Statistical analysis of the results involved firstly a within group comparison of the cytokine means between time points T₁ and T₂. We obtained two samples (T₁ and T₂) from 15/16 of controls and 14/17 of periodontitis patients; consequently, this first comparison involved 15 and 14 participants in the healthy and periodontitis group, respectively. Since cytokine levels did not differ statistically between the two time points, the two values (T₁ and T₂ were averaged). Given the lack of statistical difference between the T₁ and T₂ levels, participants who had only contributed one sample were subsequently included in the calculation of the overall healthy and periodontitis group means.

When comparing the pre-treatment to the post-treatment levels baseline data from 10/17 periodontitis patients who returned for reevaluation were used.

**HMGB-1**

Paired t-test statistical analysis of the results showed that there were no statistically significant differences in the mean levels of HMGB-1 between T₁ and T₂ for healthy controls (2.37 ng/mL vs 2.26 ng/mL) or chronic periodontitis patients (3.35 ng/mL vs 2.65 ng/mL). Differences in the overall mean concentration of HMGB-1 (mean of first and second time point) between the two groups were analyzed using t-test. Patients with chronic periodontitis were found to have statistically significantly greater levels of
HMGB-1 than the periodontally healthy participants (3.26 ng/mL vs 2.30 ng/mL, p=0.04). Non-surgical periodontal treatment led to a decrease in the serum levels of HMGB-1 from 2.82 ng/mL to 2.57 ng/mL, which was not statistically significant. The data are presented in detail in Tables 3, 4, 5 and Figures 1, 2 and 3.

<table>
<thead>
<tr>
<th></th>
<th>T1 Mean</th>
<th>T2 Mean</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>H</td>
<td>2.37 ± 0.56 ng/mL</td>
<td>2.26 ± 0.39 ng/mL</td>
<td>0.89</td>
</tr>
<tr>
<td>CP</td>
<td>3.35 ± 0.49 ng/mL</td>
<td>2.65 ± 0.38 ng/mL</td>
<td>0.09</td>
</tr>
</tbody>
</table>

Table 3: Mean HMGB-1 values ± SEM at T₁ and T₂

Figure 1: Mean HMGB-1 levels ± SEM for each group (H, CP) at T₁ and T₂
Table 4: Overall mean levels of HMGB-1 ± SEM for H and CP

<table>
<thead>
<tr>
<th></th>
<th>H</th>
<th>CP</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall Mean</td>
<td>2.30 ± 0.27</td>
<td>3.26 ± 0.36</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Table 5: Pre and post-treatment mean levels of HMGB-1 ± SEM

<table>
<thead>
<tr>
<th></th>
<th>Pre-treatment</th>
<th>Post-treatment</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CP</td>
<td>2.82 ± 0.47 ng/mL</td>
<td>2.57 ± 0.42 ng/mL</td>
<td>0.7</td>
</tr>
</tbody>
</table>
S100A12

No statistically significant differences were found in the concentration of S100A12 between different time points. For the healthy group the mean concentrations at time point T1 and time point T2 were 148.9 ng/mL and 137.2 ng/mL respectively and for the periodontitis group the mean concentrations were 157.5 ng/mL and 115.0 ng/mL respectively. The overall mean S100A12 levels were greater in the periodontitis group but not statistically different than the controls (163.3 ng/mL vs 136.8 ng/mL, p=0.20).

Subgroup analysis and comparison of the mean S100A12 levels of the patients who underwent treatment before and after scaling and root planing showed that the differences were not statistically significant (1276.7 ng/mL vs 892.2 ng/mL, p=0.12) but there was a
trend for improvement. The above data are also presented in Tables 6, 7, 8 and Figures 4, 5 and 6.

<table>
<thead>
<tr>
<th></th>
<th>T1 Mean</th>
<th>T2 Mean</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>H</td>
<td>148.9 ± 32.6 ng/mL</td>
<td>137.2 ± 20.1 ng/mL</td>
<td>0.79</td>
</tr>
<tr>
<td>CP</td>
<td>157.5 ± 28.6 ng/mL</td>
<td>115.0 ± 13.7 ng/mL</td>
<td>0.14</td>
</tr>
</tbody>
</table>

Table 6: Mean S100A12 values ± SEM at T₁ and T₂

![Figure 4: Mean S100A12 levels ± SEM for each group (H, CP) at T₁ and T₂](image)

<table>
<thead>
<tr>
<th></th>
<th>H</th>
<th>CP</th>
<th>p-value/P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall Mean</td>
<td>136.8 ± 17.3 ng/mL</td>
<td>163.3 ± 24.1 ng/mL</td>
<td>0.20</td>
</tr>
<tr>
<td>Overall Median</td>
<td>150.3 ng/mL</td>
<td>140.3 ng/mL</td>
<td>0.60</td>
</tr>
</tbody>
</table>

Table 7: Overall mean levels ± SEM and median levels of S100A12 for H and CP
Figure 5: Overall mean levels of S100A12 ± SEM for H and CP

<table>
<thead>
<tr>
<th></th>
<th>Pre-treatment</th>
<th>Post-treatment</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CP Mean</td>
<td>127.7 ± 21.8 ng/mL</td>
<td>89.2 ± 16.2 ng/mL</td>
<td>0.12</td>
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</tbody>
</table>

Table 8: Pre and post-treatment mean levels of S100A12 ± SEM
Statistical analysis using paired t-test did not reveal any statistically significant differences within groups from T1 and T2 for either controls (76.7 ng/mL and 82.7 ng/mL) or patients (99.7 ng/mL and 95.6 ng/mL). Parametric analysis between the overall means of each group was not possible due to lack of data normality. As a result both non-parametric analysis and parametric analysis after log transformation and normalization of data were performed, which showed that the patient group had higher overall mean/median levels of chemerin compared to the healthy group (median of 91.0 ng/mL vs 73.6 pg/mL with P=0.015). Although the mean levels of chemerin were reduced following treatment the differences were not statistically significant (99.9 ng/mL vs 91.8 ng/mL, p=0.25). Tables 9, 10 and 11 and Figures 7, 8 and 9 illustrate the above data.
<table>
<thead>
<tr>
<th></th>
<th>T1 Mean</th>
<th>T2 Mean</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>H</td>
<td>76.7 ± 7.5 ng/mL</td>
<td>82.7 ± 5.8 ng/mL</td>
<td>0.36</td>
</tr>
<tr>
<td>CP</td>
<td>99.7 ± 6.4 ng/mL</td>
<td>95.6 ± 5.5 ng/mL</td>
<td>0.29</td>
</tr>
</tbody>
</table>

Table 9: Mean levels of Chemerin ± SEM at T₁ and T₂

![Figure 7](image)

Figure 7: Mean Chemerin levels ± SEM for each group (H, CP) at T₁ and T₂

<table>
<thead>
<tr>
<th></th>
<th>H</th>
<th>CP</th>
<th>p-value/P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall Mean</td>
<td>79.7 ± 5.6 ng/mL</td>
<td>95.2 ± 4.9 ng/mL</td>
<td>0.04</td>
</tr>
<tr>
<td>Overall Median</td>
<td>73.6</td>
<td>91.0</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Table 10: Overall mean levels of Chemerin ± SEM for H and CP
**Figure 8**: Overall mean levels of Chemerin ± SEM for H and CP

<table>
<thead>
<tr>
<th></th>
<th>Pre-treatment</th>
<th>Post-treatment</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CP Mean</td>
<td>99.9 ± 5.7 ng/mL</td>
<td>91.8 ± 6.0 ng/mL</td>
<td>0.26</td>
</tr>
</tbody>
</table>

*Table 11*: Pre and post-treatment mean levels of Chemerin ± SEM
Figure 9: Mean levels of Chemerin ± SEM before and after treatment

BAFF

The mean levels of B-cell activating factor were found to be similar between $T_1$ and $T_2$ for both healthy controls (1150.0 pg/mL vs 1220.6 pg/mL) and periodontitis patients (1270.9 pg/mL vs 1244.4 pg/mL) when paired t-test analysis was performed. Overall, the mean levels of BAFF were higher for periodontitis patients but the difference was not statistically significant (1267.8 pg/mL vs 1176.5 pg/mL; $p=0.16$). In terms of patients' response to treatment, no statistically significant differences were found between pre and post-treatment levels (1250.1 pg/mL vs 1147.2 pg/mL) although the post treatment levels approached the levels of the healthy group. The results are presented in Tables 12, 13, 14 and Figures 10, 11 and 12.
<table>
<thead>
<tr>
<th></th>
<th>T1 Mean</th>
<th>T2 Mean</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>H</td>
<td>$1150.0 \pm 47.9$ pg/mL</td>
<td>$1220.6 \pm 57.6$ pg/mL</td>
<td>0.58</td>
</tr>
<tr>
<td>CP</td>
<td>$1270.9 \pm 58.9$ pg/mL</td>
<td>$1244.4 \pm 60.0$ pg/mL</td>
<td>0.18</td>
</tr>
</tbody>
</table>

Table 12: Mean BAFF values ± SEM at T₁ and T₂

Figure 10: Mean BAFF levels ± SEM for each group (H, CP) at T₁ and T₂

|          | H                | CP                | p-value/P value |
|----------|------------------|------------------|----------------|---------|
| Overall  | $1176.5 \pm 44.5$ pg/mL | $1267.8 \pm 45.5$ pg/mL | 0.16          |

Table 13: Overall mean levels of BAFF ± SEM for H and CP
Figure 11: Overall mean levels of BAFF ± SEM for H and CP

<table>
<thead>
<tr>
<th></th>
<th>Pre-treatment</th>
<th>Post-treatment</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CP Mean</td>
<td>1250.1 ± 74.7 pg/mL</td>
<td>1147.3 ± 40.2 pg/mL</td>
<td>0.24</td>
</tr>
<tr>
<td>CP Median</td>
<td>1215.6 pg/mL</td>
<td>1147.4 pg/mL</td>
<td>0.23</td>
</tr>
</tbody>
</table>

Table 14: Pre and post-treatment mean levels of BAFF ± SEM
For FGF-21 no differences were noted in the intragroup systemic levels between the two time points. For controls, the serum levels of FGF-21 varied from 144.7 pg/mL to 143.8 pg/mL. The respective means for the periodontitis group were 260.9 pg/mL and 185.25 pg/mL. Due to failure to pass the normality test non-parametric analysis (signed rank test) was used, which yielded a median of 133.8 pg/mL and 182.8 pg/mL for T₁ and T₂ respectively. Comparison of the overall means of healthy subjects and periodontal patients failed to reach statistical significance. The respective means were 142.5 pg/mL vs 216.7 pg/mL and data was analyzed using the Mann-Whitney U-test. Non-parametric analysis showed no statistically significant differences. The mean levels of FGF-21 before and after treatment were 236.0 pg/mL and 438.9 pg/mL respectively. The above data can be found in Tables 15, 16, 17 and Figures 13, 14 and 15.
<table>
<thead>
<tr>
<th></th>
<th>T1 Mean</th>
<th>T2 Mean</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>H</td>
<td>144.7 ± 29.1 pg/mL</td>
<td>143.8 ± 32.0 pg/mL</td>
<td>0.96</td>
</tr>
<tr>
<td>CP Mean</td>
<td>260.9 ± 104.9 pg/mL</td>
<td>185.3 ± 23.8 pg/mL</td>
<td>0.47</td>
</tr>
<tr>
<td>CP Median</td>
<td>133.8 pg/mL</td>
<td>182.8 pg/mL</td>
<td>0.76</td>
</tr>
</tbody>
</table>

Table 15: Mean ± SEM and median FGF-21 at T₁ and T₂

![Figure 13: Mean FGF-21 levels ± SEM for each group (H, CP) at T₁ and T₂](image)

<table>
<thead>
<tr>
<th></th>
<th>H</th>
<th>CP</th>
<th>p-value/P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall Median</td>
<td>114.9 pg/mL</td>
<td>153.5 pg/mL</td>
<td>0.05</td>
</tr>
<tr>
<td>Overall Mean</td>
<td>142.5 ± 27.5 pg/mL</td>
<td>216.7 ± 48.3 pg/mL</td>
<td>0.20</td>
</tr>
</tbody>
</table>

Table 16: Overall mean levels ± SEM and median levels of FGF-21 for H and CP
Figure 14: Overall mean levels of FGF-21 ± SEM for H and CP

<table>
<thead>
<tr>
<th></th>
<th>Pre-treatment</th>
<th>Post-treatment</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CP Mean</td>
<td>236.0 ± 77.4 pg/mL</td>
<td>438.9 ± 238.4 pg/mL</td>
<td>0.25</td>
</tr>
<tr>
<td>CP Median</td>
<td>154.7 pg/mL</td>
<td>224.2 pg/mL</td>
<td>0.23</td>
</tr>
</tbody>
</table>

Table 17: Pre and post-treatment mean levels ± SEM and median levels of FGF-21
Within group analysis using paired t-test showed that there was no significant difference between time point $T_1$ and time point $T_2$ in healthy subjects (0.526 µmol/L vs 0.547 µmol/L). Among patients, however, differences between time points 1 and 2 were found statistically significant (0.501 µmol/L vs 0.583 µmol/L, p=0.01). The total mean levels of ADMA were greater in the periodontitis group (0.560 µmol/L) compared to the control group (0.535 µmol/L) but the difference was not statistically significant (p=0.61). In response to periodontal treatment, the mean ADMA levels decreased slightly but not statistically significantly (from 0.590 µmol/L to 0.579 µmol/L). A synopsis of the findings can be seen on Tables 18, 19 and 20 and Figures 16, 17 and 18.
<table>
<thead>
<tr>
<th></th>
<th>T1 Mean</th>
<th>T2 Mean</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>H</td>
<td>$0.526 \pm 0.038$ µmol/L</td>
<td>$0.547 \pm 0.050$ µmol/L</td>
<td>0.74</td>
</tr>
<tr>
<td>CP</td>
<td>$0.501 \pm 0.036$ µmol/L</td>
<td>$0.583 \pm 0.044$ µmol/L</td>
<td>0.01</td>
</tr>
</tbody>
</table>

**Table 18**: Mean ADMA values ± SEM at $T_1$ and $T_2$

![Figure 16: Mean ADMA levels ± SEM for each group (H, CP) at $T_1$ and $T_2$](image)

<table>
<thead>
<tr>
<th></th>
<th>H</th>
<th>CP</th>
<th>p-value/</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall Mean</td>
<td>$0.535 \pm 0.031$ µmol/L</td>
<td>$0.560 \pm 0.037$ µmol/L</td>
<td>0.61</td>
</tr>
</tbody>
</table>

**Table 19**: Overall mean levels of ADMA +/- SEM for H and CP
Figure 17: Overall mean levels of ADMA ± SEM for H and CP

<table>
<thead>
<tr>
<th></th>
<th>Pre-treatment</th>
<th>Post-treatment</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CP Mean</td>
<td>0.590 ± 0.052</td>
<td>0.579 ± 0.046</td>
<td>0.76</td>
</tr>
</tbody>
</table>

Table 20: Pre and post-treatment mean levels of ADMA ± SEM

Figure 18: Bar graphs of mean levels of ADMA ± SEM before and after treatment
Chapter 4

Discussion

The purpose of the present study was to measure the systemic levels of a number of inflammatory mediators in patients with chronic periodontitis and compare them to periodontally healthy controls. Additionally, it was investigated whether there is a significant variability in the levels of these mediators in the short term. Finally, an attempt to evaluate the effect of non-surgical periodontal treatment on their levels was performed. Our findings indicated that periodontitis patients have statistically significantly higher systemic levels of HMGB-1 and chemerin compared to periodontally healthy subjects. Therefore, for two of the mediators, i.e., HMGB-1 and chemerin, the results of this study are consistent with our working hypothesis. No statistically significant intragroup differences were found between time points for any of the mediators measured. The levels of the inflammatory mediators investigated did not change statistically significantly following non-surgical periodontal treatment but the small sample size (n=10) and the potential need for further treatment for some of the patients does not allow for a safe interpretation of the results. A more detailed discussion of the results follows.
Demographics

The two groups of patients were well matched and the average age of the periodontitis group is consistent with studies based on NHANES data, which indicate a higher prevalence of periodontal disease with increasing age.\textsuperscript{107} The fact that slightly more males than females were present in the periodontitis group is consistent with previous epidemiologic studies as well.\textsuperscript{107}

HMGB-1

To our knowledge this is the first study to investigate the systemic levels of HMGB-1 in patients with chronic periodontitis.

HMGB-1 can be found in the nucleus of eukaryotic cells from which it can be released following cell apoptosis. HMGB-1 amplifies the inflammatory state by increasing adhesion of monocytes, augmenting neutrophil migration, adhesion and bactericidal capability and by inducing the release of a series of pro-inflammatory cytokines.

In the present study it was shown that the overall mean serum levels of HMGB-1 in the periodontitis group were statistically significantly greater than the HMGB-1 levels in the periodontally healthy group (approximately 3.3 ng/mL vs 2.3 ng/mL). The only available study in HMGB-1 in periodontal literature compared the GCF levels of chronic periodontitis patients to the GCF levels of patients with aggressive periodontitis and
healthy controls. In that study the differences were found to be statistically significantly
greater in patients compared to controls (1.9 ng/mL vs 0.02 ng/mL), however the
magnitude of the difference was much greater than in our study (3.3 ng/mL vs 2.3 ng
/mL).\textsuperscript{108} It is not possible to comment on differences in the methodologies followed in
each study. First and foremost, in our study we analyzed the systemic levels of HMGB-1
in serum, whereas in the study by Xie the samples were obtained from GCF. Since
HMGB-1 has been found to be greatly produced by periodontal ligament cells and pocket
epithelium in response to inflammation \textsuperscript{109, 110}, it is reasonable to hypothesize that the
differences in the GCF levels of HMGB-1 between diseased and controls would be more
pronounced than in serum. Furthermore, in the present study the majority of the chronic
periodontitis patients exhibited advanced periodontal disease. There is no information on
the severity of periodontal disease of patients recruited in the aforementioned GCF study;
additionally, there is no information on the demographics of their two groups. In the
present study both groups were well balanced with respect to age and gender. In response
to treatment, the levels of HMGB-1 did not change statistically although there was a
trend for decrease. This could be attributed to inadequate reduction of local inflammation
either due to insufficient instrumentation or due to the need for additional surgical
intervention after completion of scaling and root planing. The high attrition rate (only
10/17 chronic periodontitis patients underwent periodontal treatment and returned for
reevaluation) may have also reduced the possibility of identifying statistically significant
differences through statistical analysis.
In terms of serum levels of HMGB-1 reported for other inflammatory and/or autoimmune conditions results vary greatly. Indicatively, in patients with systemic lupus erythematosus one study showed that serum HMGB-1 concentration was 27ng/mL vs 0 ng/mL in healthy controls. Another study performed on patients with juvenile systemic lupus found that patients had a mean serum concentration of HMGB-1 of 22.5 ng/mL vs 1.38 ng/mL for healthy controls. Conversely, in Sjogren's syndrome the differences in the serum levels of HMGB-1 between cases and controls were found to be more similar to the present study (1.79ng/mL vs 1.46ng/mL). Collectively, it seems that the serum levels of HMGB-1 found in our study are within the range reported in the literature for medical conditions.

**S100A12**

This is the second study in periodontal literature that evaluated the systemic levels of S100A12 in patients with chronic periodontitis. S100A12 is an alarmin, which has been shown to have strong inflammatory functions by enhancing neutrophil adhesion and stimulating the production of potent pro-inflammatory cytokines.

The present results showed that there was no statistically significant difference in the serum levels of S100A12 between chronic periodontitis patients and healthy controls although the mean levels of S100A12 were higher in the chronic periodontitis group compared to control (163 ng/ml vs 137 ng/mL). Following periodontal treatment the
levels of S100A12 decreased by 39 ng/mL but again the difference was not found to be statistically significant. The only available study that explored the systemic levels of S100A12 in chronic periodontitis patients found statistically significant differences between those patients and healthy controls (113 ng/mL vs 32 ng/mL).\(^{51}\) The greater mean S100A12 values found in our study compared to the study by Pradeep and coworkers may be attributed to disease severity. In the present study most of the patients were diagnosed with moderate-severe periodontitis. Although there is no detailed information provided in the Pradeep et al. study, their inclusion criteria would allow for recruitment of patients with localized disease only.

When comparing our results to the means of S100A12 found in various medical conditions it is hard to draw safe conclusions due to the range of values reported. For instance, two studies showed that in patients with diabetes mellitus type 2 the mean levels of S100A12 were approximately twice as high as in controls. However, in one study the calculated means were 19.6 ng/mL vs 8.1 ng/mL for the diabetic and the control group respectively, whereas in the other study the corresponding values were approximately 400 ng/mL vs 200 ng/mL.\(^{113,114}\) In patients with acute exacerbations of cystic fibrosis the levels of S100A12 were found to be 225 ng/mL compared to 64 ng/mL in the controls. Similar results in the average concentration of S100A12 in healthy controls were reported in a study focusing on obstructive sleep apnea (78 ng/mL in controls vs 132 ng/mL in diseased) and a study focusing on rheumatoid arthritis (60 ng/mL in controls vs 340 ng/mL in arthritis patients).\(^{115,116}\) Contrary to the latter study, healthy control S100A12 levels were found significantly greater in a study focusing on a type of systemic
idiopathic arthritis (272 ng/mL vs 578 ng/mL).\textsuperscript{117} Overall, our results for both the chronic periodontitis and the healthy control groups are within the range of values reported in the literature. The difference between our groups appears to have a relatively lower magnitude compared to more generalized and/or acute inflammatory and autoimmune diseases.

\textit{Chemerin}

The present study is the first one to explore the systemic levels of chemerin in patients with chronic periodontitis. The serum levels of chemerin were found to be statistically significantly greater in the chronic periodontitis group compared to the periodontally healthy group. To the best of our knowledge there has not been a previous study evaluating the systemic levels of chemerin in patients with periodontal disease.

The only study available to date is a study by Ozcan et al., which investigated the salivary levels of visfatin, progranulin and chemerin in periodontally healthy, gingivitis and chronic periodontitis groups.\textsuperscript{118} According to their results, the levels of chemerin were statistically significantly greater in the saliva of patients with periodontitis compared to patients with gingivitis and healthy controls. No differences were noted between the gingivitis and healthy controls. There were no differences in the chemerin levels related to patient gender. A positive correlation was found between salivary chemerin levels and G.I., P.D. and CAL.\textsuperscript{118} It is not possible to compare the results of the study by Ozcan et
al. to the present one, since there seems to be a lack of information in the literature regarding the correlation between serum and salivary levels of chemerin in patients with periodontitis. The difference in the salivary levels of chemerin between the periodontitis group and the other two groups were almost double (0.084 ng/mL versus 0.042 ng/mL for both gingivitis and healthy control groups). In the present study the differences between the two groups albeit statistically significant were not as pronounced (medians were 91 ng/mL for chronic periodontitis and 74 ng/mL for periodontally healthy patients). This may be related indirectly to a difference in the average age of patients recruited in the two studies. In the study by Ozcan et al., the average ages of controls and patients with chronic periodontitis were 36 and 33 years respectively, whereas in the present study the corresponding ages were 48 and 46.9 years. With increasing age there is a higher possibility that study recruits from both groups may have had additional medical conditions, which were not reported or had not been diagnosed. Such conditions could have contributed to an elevation in the chemerin levels with a more profound effect on the control group thus concealing the difference between groups attributed to periodontal inflammation. Another possible explanation for the disparity in the magnitude of the differences in the chemerin levels between groups reported in the two studies might be the inclusion criteria used. In the present study only patients diagnosed with chronic periodontitis were included, whereas in the study by Ozcan et al. there was no distinction between aggressive and chronic periodontitis in their inclusion criteria. Although smoking was not part of the present study exclusion criteria, it is likely that smoking status did not affect our results, given the limited number of smokers included and the
reported lack of correlation between the systemic levels of chemerin and smoking status in patients with chronic obstructive pulmonary disease.\textsuperscript{119}

In the medical literature a number of studies have investigated the systemic levels of chemerin in autoimmune and metabolic conditions. Patients with diabetes Type 2 were found to have statistically significantly higher peripheral levels of chemerin compared to participants with normal glucose tolerance (187 ng/mL vs 169 ng/mL). Although these levels are higher than the findings of the present research, the numerical difference between the two groups (diabetic patients vs controls) is very similar to the intergroup difference calculated in the present study (periodontitis vs controls).\textsuperscript{120} Similarly, in a recent study comparing the systemic levels of chemerin between osteoporosis patients and controls the intergroup difference was approximately 16 ng/mL (87.3 ng/mL vs 71.1 ng/mL respectively).\textsuperscript{121} In fact their results are numerically very close to the present study's ones. Another recent observational study found that patients with rheumatoid arthritis had mean serum levels of chemerin of 114 ng/mL, which again appear to be numerically close to the present study's results (91ng/mL for the periodontitis group).\textsuperscript{122} Overall, the results of the present research seem to fall within the range of results that have been previously reported in the medical literature.
BAFF

There are only very few studies that have investigated the systemic levels of BAFF in patients with chronic periodontitis. B-cell activating factor (BAFF) has been found to be important for the maturation, activation, proliferation and differentiation of a number of cells involved in innate and acquired immunity such as neutrophils, B-cells and T-cells.\textsuperscript{67, 75, 76}

The present findings showed that the levels of BAFF in plasma, albeit greater in the chronic periodontitis group (1,268 pg/mL vs 1,177 pg/mL), did not reach statistical significance. After treatment, there was a slight decrease in the levels of BAFF that failed to reach statistical significance. Interestingly, the post treatment levels of BAFF appeared to be close to the mean levels of BAFF in the healthy group of patients (1,147 pg/mL vs 1,176 pg/mL).

In periodontal literature one group of researchers showed that there was a statistically significant difference between the two groups with periodontitis patients having a median of approximately 3,500 pg/mL compared to 2,000 pg/mL in healthy controls. In another study by the same research group the median values of BAFF were found to be 2,310 pg/mL for chronic periodontitis patients.\textsuperscript{82, 123} The differences in the results between these two studies and the present study might be attributed to differences in the assays used. In the present study a commercially available ELISA was used, whereas in the study by Gumus et al. an in house assay was prepared.\textsuperscript{82} Additionally, differences in levels of tissue inflammation may be have had an impact, since in their study patients
characterized by extremely inflamed tissues (Bleeding of probing of approximately 100%). Another study comparing the salivary levels of BAFF in patients with periodontitis and primary Sjoegren's syndrome compared to patients with periodontitis and xerostomia found salivary levels of BAFF of 7,400 pg/mL vs 1,000 pg/mL. Although the levels of BAFF were also measured in the serum of these patients and they were analyzed in correlation to the salivary levels, no numerical data is included in the article, which prevents any direct comparison between that study and the present one.\textsuperscript{81}

In medical literature there is a great number of studies exploring the systemic levels of BAFF in autoimmune conditions. In rheumatoid arthritis the serum concentration of BAFF was found to be statistically significantly greater than healthy controls (700 pg/mL vs 500 pg/mL).\textsuperscript{124} Another study on children's myasthenia gravis showed a statistically significant difference between patients and controls (1733 pg/mL vs 1183 pg/mL) with their control values being close to the control valued of the present study (1183 pg/mL vs 1177 pg/mL). Lower healthy control averages (725 pg/mL) than in the present study were reported by Zhao et al on a study on autoimmune hemolytic anemia, where patients were found to have significantly greater levels of BAFF (1383 pg/mL) over controls.\textsuperscript{73}

In summary, our results showed no differences between the two groups and our values were within the range of values reported in the literature.
Up to now, to our knowledge, there are no publications in the periodontal literature that have explored the concentration of FGF-21 in either GCF or serum/plasma of patients with periodontitis. FGF-21 is a protein, which has been mainly associated with metabolic syndrome.

The present study found no statistically significant difference between serum levels of FGF-21 in chronic periodontitis patients compared to healthy controls (154 pg/mL vs 115 pg/mL respectively). FGF-21 is a protein, which has been mainly associated with metabolic syndrome. Due to its implication in metabolic syndrome FGF-21 levels have been investigated primarily in patients with diabetes mellitus. One study showed that the concentration of FGF-21 was significantly higher in diabetic patients with subclinical atherosclerosis vs diabetic controls (261 pg/mL vs 145 pg/mL respectively). The value for the diabetic control group is very similar to the present study periodontitis group (145 pg/mL vs 154 pg/mL respectively). In another study on gestational diabetes mellitus it was shown that the patient group had statistically significantly higher serum levels of FGF-21 compared to the healthy control group (125 pg/mL vs 68 pg/mL)\textsuperscript{125}. Compared to the present results, the levels of FGF-21 in the control group of the latter study appear lower. This discrepancy could be attributed to differences in the mean age of healthy controls. The mean age of controls in the present research was 48 years as opposed to a mean age of 29 in the diabetic patient study; it has been shown that incremental increases in age correspond to increases in the circulating levels of FGF-21.\textsuperscript{126} Differences in age
could also explain the higher levels of FGF-21 reported for healthy controls in a cross-sectional study on patients with paroxysmal atrial fibrillation, where healthy individuals had a mean serum FGF-21 of 144 pg/mL vs 250 pg/mL for patients. In that study, the mean ages of patients and controls were 58 and 66 years, respectively, which are greater compared to the present study averages (48 and 47 years respectively). In another study, FGF-21 levels were compared between controls and patients with non-mitochondrial neuromuscular disease (disease of neurologic or muscular origin); the serum levels of FGF-21 were found to be approximately 222 pg/mL, which was statistically significantly higher than the FGF levels in healthy controls (83 pg/mL).

One interesting observation the present study is the change of FGF-21 levels in the subgroup of patients who received treatment. Although the differences were found to be non statistically significantly different, compared to all other biomarkers investigated, the median values showed an increase. This could be largely related to a small patient sample size (n=10). However, an increase in the levels of FGF-21 following therapy has been previously reported following treatment of diabetes. That increase was explained on the grounds of improved metabolic control of the patients in response to the received treatment.
To the best of our knowledge, there are no published studies that have investigated the ADMA levels in periodontal disease. Asymmetric dimethylarginine is a naturally occurring amino acid, which has been associated with various autoimmune and inflammatory diseases due to its inhibitory action on NOS (nitrous oxide synthase). In the present study no statistically significant differences were found in the levels of ADMA in periodontal disease patients compared to healthy controls (p=0.6). The mean values for both groups numerically were very similar (0.560 µmol/L vs 0.535 µmol/L). Periodontal treatment did not seem to significantly change the levels of ADMA (decrease of 0.011 µmol/L).

In the dental literature there are two publications from Cotti et al, which measured the levels of ADMA in apical periodontitis.\textsuperscript{103, 104} That research group found significantly greater ADMA levels in patients with apical periodontitis versus matched healthy controls (0.725 µmol/L vs 0.650 µmol/L). Since the present study evaluated a different oral disease than Cotti's it is not possible to attempt a direct comparison between the two.

ADMA levels have been explored in a variety of medical conditions. In patients with rheumatoid arthritis plasma levels of ADMA were not found to be statistically significantly different than controls. In the same study ADMA levels in peripheral artery occlusive disease (PAOD) were found higher than in coronary artery disease (CAD) (0.529 µmol/L vs 0.505 µmol/L).\textsuperscript{131} Conversely, a different research group found that anti-TNF-α treatment led to a reduction of ADMA levels from 0.530 µmol/L to 0.440
µmol/L in patients with rheumatoid arthritis. When the ADMA concentration was evaluated in patients with glaucoma, significantly higher levels compared to controls (0.640 µmol/L vs 0.600 µmol/L respectively) were found. Statistical significant differences in systemic ADMA concentrations were also found between patients in chronic hemodialysis and healthy controls (0.53 µmol/L vs 0.35 µmol/L). The most interesting observation derived from the medical literature is that the systemic levels of ADMA in patients and controls are often numerically very close. As a result, a large sample size is needed in order to obtain the statistical significance for any differences found between groups. In the present study, the total number of recruits was too small for results to reach statistical significance.

**Limitations of the present study - Future suggestions**

One of the first limitations of the present study is the limited sample size. In comparison to our study, the majority of the medical studies that investigated the same inflammatory mediators utilized a greater pool of patients. Furthermore, out of the 17 periodontitis patients only 10 completed the non-surgical treatment. This relatively high attrition rate could be the result of limited financial capacity of the patients screened in the OSU College of Dentistry. At the same time, the low educational background of many of these patients impairs the providers' efforts to make them aware of the importance of periodontal treatment. In order to overcome these shortcomings, multi-centered studies should be organized in the future, that will allow for an increased patient sample size.
Additionally, emphasis should be placed on the significance of periodontal treatment for oral health by use of educational aides, such as videos and leaflets. This could potentially reduce the attrition rates and increase the available sample size of patients with completed treatment.

Another limitation of the present study is the lack of control over treatment providers. Since periodontal treatment was not provided as part of the study itself, in which case a single treatment provider could have been used, the patients were treated by a number of treatment providers. Moreover, the experience levels of the different providers differed (3rd year dental students, 4th year dental students, periodontology residents), which may have had an influence on the efficiency of the treatment. In the future, it would be advisable to implement a single treatment provider with a good level of experience (i.e. periodontal resident) by utilizing a longitudinal randomized control clinical study design.

Last but not least, in the present research we evaluated the effect of solely non-surgical periodontal treatment on the systemic levels of selected mediators. Non-surgical treatment is an important step in the periodontal treatment plan but not always an endpoint. Sometimes surgical intervention is needed in order to control the disease and/or restore periodontal health. Future studies should follow up patients even after surgical treatment (if it is part of the overall periodontal treatment). This would allow for an evaluation of the influence of the overall periodontal treatment on the systemic levels of inflammatory mediators investigated as opposed to evaluating the influence of non-surgical treatment alone.
Chapter 5

Conclusion

Our study showed that the peripheral levels of HMGB-1 and chemerin were statistically significantly greater in patients with chronic periodontitis compared to healthy controls. The levels of S100A12, BAFF, FGF-21 and ADMA although increased in the group of periodontitis patients failed to reach statistical significance. Non-surgical periodontal treatment decreased the levels of all inflammatory mediators except for FGF-21 but the changes were not found to be statistically significant.

In contrast to the medical literature, there is a paucity of evidence regarding the systemic levels of novel inflammatory mediators in periodontitis. The present work lays the foundation for future studies that will explore the systemic levels of such mediators. Multi-centered studies with a greater sample size and calibrated treatment providers, including defined treatment end-points, will be able to demonstrate whether these markers are truly important in disease pathogenesis and in the level of systemic inflammatory response developed against local infection or not.

A potential identification of systemic biomarkers that can indicate the presence of periodontitis or reflect the patient's response to treatment could prove to be of great use in
the diagnosis, treatment, monitoring as well as prognosis of periodontitis by offering non-invasive alternatives to traditional screening modalities and allowing for an improved prediction of the results of our treatment.
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