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Degree and Date to be conferred: M.S., May 2024

Education

2021- Present	**University of Maryland**
	Baltimore, MD
	Certificate in Advanced Prosthodontics
2021- 2024	**University of Maryland**
	Baltimore, MD
	Master of Biomedical Sciences
2017- 2021	**Temple University - Kornberg School of Dentistry**
	Philadelphia, PA
	Doctor of Dental Medicine (D.M.D)
2013-2017	**Temple University, College of Science and Technology**
	Philadelphia, PA
	Bachelor of Science in Biology; Healthcare Management minor

Peer Reviewed Publications

Journal of Prosthodontics

Balshi TJ, Wolfinger GJ, Pellecchia R, Reiger W, Blakely JW, Balshi SF, **Kachlan MO**. 9-Year Follow-Up on Maxillofacial Implant-Supported Framework Designed to Accommodate Childhood Growth. J Prosthodont. 2022 Aug;31(7):551-561.

Journal of Prosthodontics

Kachlan MO, Yang J, Balshi TJ, Wolfinger GJ, Balshi SF. Incidental Findings in Cone Beam Computed Tomography for Dental Implants in 1002 Patients. J Prosthodont. 2021 Oct;30(8):665-675

Contributions to Publications:

McReynolds DE, Moorthy A, Moneley JO, Jabra-Rizk MA, Sultan AS. Denture stomatitis-An interdisciplinary clinical review. J Prosthodont. 2023 Aug;32(7):560-570.

Provided photos of denture stomatitis and old infected denture prostheses with heavy biofilm, demonstrating dentures can act as fomites reservoirs for future inoculation and infection of denture stomatitis.

Professional Experience

2016- Present **Prosthodontics Intermedica (Pi) Dental Center**

Fort Washington, PA

Research Associate

Attended continuing education course training:

April 29, 2016 AvaDent Clinical Training

May 16, 2016 The severely atrophic maxilla: Stabilizing implants in the pterygomaxillary & zygoma implants

July - Dec 2018 **Department of Anatomy and Cell Biology, Lewis Katz School of Medicine at Temple University**

Philadelphia, PA

Gross Anatomy Dissector

Performed 100+ hours of dissection procedures on 35 cadavers that were used in the gross anatomy courses at the medical school. Dissections included laminectomies and dissection of the facial nerve branches.

2018-2020 **Temple University - Kornberg School of Dentistry**

Philadelphia, PA

Examiner Assistant for ADEX licensing exam

Worked directly with the examiners to ensure the ADEX examination runs efficiently and smoothly for DMD candidates.

Professional Memberships/Affiliations

2014-Present **Academy of Osseointegration**

Member

2018-Present **The American College of Prosthodontists**

Member

2017-Present **American Dental Association**

Member

Community Service

Sept 21, 2019 ***Give Kids a Smile Day***

Pediatric clinic, Kornberg School of Dentistry

Philadelphia, Pennsylvania

Provided preventative care (cleanings and sealants) to uninsured and underserved children in Philadelphia.

2015-2016 **ESL Teacher**

Nationalities Service Center

Philadelphia, PA

Volunteered to teach English bi-weekly to adult immigrants and refugees.

2016 **Temple University - Center for American Language and Culture**

Philadelphia, Pennsylvania

ESL Teacher

Taught bi-weekly English classes that included activities based on pronunciation and writing skills while implementing visual and audio learning techniques to international students.

Honors & Awards

Cary Klimen, D.D.S, Ethics Award **May 2021**

Presented to the student who has exhibited personal values and actions that demonstrate integrity and ethics.

American Academy of Implant Dentistry **May 2021**

Certificate of recognition for outstanding achievement, both academically and clinically, in implant dentistry as recommended by Temple University Maurice H. Kornberg School of Dentistry.

American Academy of Oral and Maxillofacial Radiology **May 2021**

Certificate of recognition for greater interest and accomplishment in Oral and Maxillofacial Radiology.

POSTER PRESENTATIONS:

American Academy of Fixed Prosthodontics **February 2024**

"Prosthetically driven implant placement and prosthodontic treatment using dynamic navigation"

The American College of Prosthodontists 53rd annual meeting **October 2023**

"Prosthetically driven implant placement and prosthodontic treatment using dynamic navigation"

LICENSURE & CERTIFICATION:

Maryland & Pennsylvania Dental Licenses

Phlebotomy

CPR Certified

Abstract

Mamdouh Omar Kachlan, Master of Science, 2024

Thesis Directed by: Dr. Radi Masri, BDS, MS, PhD

Professor & Director, Advanced Education Program in Prosthodontics

Director, Division of Prosthodontics

Department of Advanced Oral Sciences and Therapeutics

University of Maryland, School of Dentistry

Title of Thesis: Saliva Levels of IL-18 in healthy and peri-implantitis patients

Purpose: Peri-implantitis is a common problem that occurs in 9.25% in functioning implants and approximately 20% of patients. Our understanding of the etiology of Peri-implantitis is limited. Understanding molecular mechanisms associated with peri-implantitis may help in developing and improving treatments for peri-implantitis.

The purpose of this study was to assess the levels of IL-18 in saliva of healthy patients and patients diagnosed with peri-implantitis.

Materials and Methods: Institutional Review Board approval was obtained. Unstimulated saliva was collected from a total of 24 subjects (peri-implantitis n=14, healthy n=10). Saliva was collected from subjects using 15 ml tubes every minute for 5 minutes. Collected saliva were then centrifuged for 5 minutes at 10,000 x g. The aliquot layer was collected and immediately stored at -80°C until analysis. The concentration level of IL-18 was measured using high-sensitivity enzyme-linked immunoabsorbent

assays (ELISA). All statistical analyses were performed using Microsoft® Excel®. Statistical comparisons were tested for normality followed by the Mann Whitney U test.

Results: Twenty-four subjects with 33 implants were analyzed. Twenty-two implants were diagnosed with peri-implantitis, while the remaining 11 were healthy controls. The Median values of IL-18 analytes were 1.92 pg/ml for peri-implantitis and 2.23 pg/ml for healthy control. The range was 8.58 pg/ml for peri-implantitis (min 0.079 pg/ml – max 8.66 pg/ml). The range was 21.26 pg/ml for healthy control (min 1.13 pg/ml – max 22.39 pg/ml). The mean was 5.47 pg/ml for healthy control and 2.61 pg/ml for peri-implantitis. The standard deviation was 6.80 pg/ml for healthy control and 2.04 pg/ml for peri-implantitis.

Conclusions: In non-smoking patients not suffering from diabetes or other inflammatory disease, it appears that the levels of IL-18 are comparable to those of healthy patients.

Saliva Levels of IL-18 in Healthy and Peri-implantitis Patients

by
Mamdouh Omar Kachlan

Thesis submitted to the Faculty of the Graduate School of the
University of Maryland, Baltimore in partial fulfillment
of the requirements for the degree of
Master of Science
2024

To my parents, Lubna and Omar. I owe you all my success.

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Chapter 1 Introduction and Background

1.1 *Edentulism*

Edentulism is defined as the loss of all natural teeth (GPT-9 2017). According to the National Center for Health Statistics, the population of edentulous individuals in the United States surpasses 36 million (Dye et al, 2015). An additional 120 million people are affected by the absence of at least one tooth (Dye et al, 2015). There are biological processes such as caries, periodontal disease, xerostomia, congenitally missing teeth, oral cancer, as well as nonbiologic factors related to dental procedures such as trauma, access to and availability of care, patient preferences and awareness, financial coverage, and treatment options that contribute to edentulism (Felton, 2009). Twenty-four percent of adults aged 65 to 74 years lost all their natural teeth in 1999-2004 (Dye et al., 2007). Forty-seven and a half percent of adults aged 45 to 74 years had moderate or severe periodontitis in 2009–10 (Dye et al., 2007). Estimates suggest that edentulism rate declines by 10 percent every decade (Marcus et al., 1996). A report indicates a 6% reduction in total edentulism between 1988 and 2002 (Beltran-Aguilar et al., 2005). This reduction in edentulism will be offset by the 79% increase in adult population older than 55 years (Douglass et al., 2002). Edentulous people are considered impaired by the World Health Organization, due to their limited capacity to eat or speak well (Felton, 2009). Studies have found that edentulous patients have a limited intake of dietary fiber and vitamins than did dentate counterparts (Hines et al., 1998; Fontijin-Tekamp et al., 1996). They are also more likely to be smokers, have poor nutrition, develop obesity, dementia, osteoporosis, diabetes, hypertension and coronary artery disease (Felton, 2009).

1.2 *Dental Implants*

Dental implants have functioned as a reliable and predictable treatment option to replace missing teeth. Several studies reported high survival rates (Ali et al., 2019). A recent study of 10,871 dental implants with up to 22 years of follow-up in 4247 patients found the cumulative survival rate at the implant level at 3, 5, 10, and 15 years was 98.9%, 98.5%, 96.8%, and 94.0%, respectively, while survival rate at the patient level was 97.4%, 96.7%, 92.5%, and 86% at 3, 5, 10, and 15 years, respectively (French et al., 2021). The incidence of peri-implant mucositis at the implant level was 9.4% at 2–3 years, 9.3% at 4 to 5 years, 12.1% at 6 to 7 years, and 11.9% at 8 to 10 years (French et al., 2021). The incidence of peri-implantitis was 2%, 2.6%, 3.2%, and 7.1% at 2 to 3, 4 to 5, 6 to 7, and 8 to 10 years, respectively (French et al., 2021). In a systematic review and meta-analysis of 47 clinical studies showing follow-up of a minimum of three years, the weighted average prevalence of peri-implantitis based on implants and subject levels was 9.25% and 19.83%, respectively. Similarly, the weighted mean prevalence of peri-implant mucositis based on implants and subject levels was 29.48% and 46.83%, respectively (Lee et al., 2017).

1.3 *Complications & Implant Failure*

Complications in implant therapy can be classified as biologic, technical, esthetic, and surgical (Paquette et al., 2006). Implant failure can occur early when osseointegration fails to occur, or late when osseointegration is lost after implant has been loaded under function (Paquette et al., 2006). Early failure is attributed to early loading, excessive micromotion, poor primary stability, poor infection control, surgical technique, poor biocompatibility of the implant material, or inefficient healing because of systemic disease (Paquette et al., 2006). In contrast, late failures occur due to anatomic and osseous factors,

such as type 4 bone quality and limited bone quantity, infection or inflammation, mechanical failure, fracture, iatrogenic failure, or patient related failure (Paquette et al., 2006). Parafunctional habits, such as clenching and bruxism, are associated with technical and prosthetic failures such as screw loosening, porcelain fracture, or prosthesis fracture. Chronic infection of the peri-implant tissues is emerging as the most common cause for late implant failure and recent studies suggest that the prevalence of peri-implant inflammation is grossly underestimated (Belibasakis et al., 2015). Recently, complications due to inflammation and bone and implant loss have been increasingly seen (Goh et al., 2017). Peri-implant inflammation, as with periodontal inflammation, results from a disruption in host-compatible/pathogenic microorganisms that may lead to two specific clinical diseases: peri-implant mucositis and peri-implantitis, resembling gingivitis and periodontitis, respectively (Mombelli et al., 1987; Esposito et al., 1997; Smeets et al., 2014). It is projected that peri-implant mucositis precedes peri-implantitis but there are no discernible characteristics or circumstances contributing to the transition of the disease. (Schwarz et al., 2018).

1.4 *Biologic Width*

The biologic width of implants and teeth are different (Zheng et al., 2020). Implants lack inter-septal periodontal fibers and a periodontal ligament (Zheng et al., 2020). Lack of periodontal ligament (PDL) leads to reduced blood supply (Zheng et al., 2020). The perpendicular orientation of collagen fibers around teeth provides a protective physical barrier from bacterial invasion, whereas in implants the fibers show a parallel pattern (Tetè et al., 2009). It is advantageous to have a +2mm in width and at least 1-1.5mm in thickness

of keratinized mucosa (KM) to minimize the chance of inflammation and recession (Berglundh et al., 2017). Elevated plaque and bleeding scores have been observed at sites with diminished keratinized gingiva (KG) (Souza et al., 2016). Additionally, patients reported higher levels of discomfort brushing at sites with KG <2 mm (Souza). In a prospective 10-year long study that examined 98 patients, the absence of KG was linked with elevated plaque accumulation, increased soft-tissue recession, and a greater number of sites necessitating an additional surgical and/or antibiotic interventions despite patients practicing adequate oral hygiene and regularly receiving supporting periodontal therapy (Roccuzzo et al., 2016).

1.5 *Peri-implant Mucositis*

Peri-implant mucositis is a reversible soft tissue inflammation of soft tissues around the dental implant (Jepsen et al., 2015). Peri-implant mucositis is an inflammatory lesion of the mucosa surrounding titanium endosseous implants and may involve erythema, swelling, bleeding on gentle probing, and suppuration (Caton et al., 2018). Bleeding on probing can be present but no bone loss further than the initial remodeling is seen. Peri-implant mucositis may precede peri-implantitis (Caton). In studies of experimental biofilm accumulation on healthy gingiva and mucosa surrounding dental implants, there was an observable onset of mucosal inflammation, including symptoms like swelling, redness, and bleeding during the period in which oral hygiene practices were discontinued, demonstrating a cause and effect relationship between biofilm accumulation and peri-implant mucositis (Pontoriero et al., 1994; Salvi et al., 2012; Meyer et al., 2017). Studies have revealed that, despite observing less plaque and biofilm accumulation at implant sites,

the peri-implant mucosa exhibited a higher proportion of bleeding sites compared to the gingiva, there was a more prominent inflammatory response at the implant sites, although subjected to a comparable bacterial exposure (Salvi et al., 2012). Cigarette smoking has been found to be associated with an increased risk of peri-implant mucositis. There is evidence suggesting that radiation therapy, diabetes mellitus, and excess cement around implant restorations may serve as risk factors for peri-implant mucositis (Karbach et al., 2009; Roos-Jansaker et al., 2006; Rinke et al., 2011; Ferreira et al., 2006; Linkevicius et al., 2013).

1.6 *Peri-implantitis*

Peri-implantitis is a pathological condition occurring in tissues around dental implants, characterized by bleeding and/or suppuration, increased probing depth, inflammation in the peri-implant mucosa and progressive loss of supporting bone evidenced by radiographs, beyond initial biological bone remodeling (Schwarz et al, 2018; Caton et al, 2018). Biologic bone remodeling is initial physiologic marginal bone loss of less than 1.5 mm during the first year after implant placement and less than 0.2 mm in the following years of function (Worthington et al., 1987; Albrektsson et al., 1986; Ramanauskaite et al., 2016). This is influenced by implant type and surface, smooth/rough border, and the introduction of a micro-gap (Hermann et al., 2000). Early bone loss detected in the initial 3-6 months is a predictor for early peri-implantitis (Windael et al., 2021). The onset of peri-implantitis can occur early within 3 years of function (Derks et al, 2016). First signs of bone loss (>0.5 mm) were seen in most implants after the second (52%) and third year (66%) in function in 70% and 81% of patients, respectively (Derks).

Peri-implant mucositis occurs in 80% of patients and 50% of implant sites, and peri-implantitis occurs between 28-56% of patients tested and 12-40% of sites (Lindhe et al., 2008). The peak rate of peri-implantitis incidence after the 7th year and the prevalence increased between 5 and 10 years in function (Pandolfi et al., 2019). In a systematic review of forty-seven manuscripts, Lee et al found peri-implantitis to affect 9.25% of implants and 19.83% of patients (Lee et al., 2017). In the absence of previous examination data, making the diagnosis of peri-implantitis challenging. The American Academy of Periodontology (AAP) in the Consensus report of workgroup 4 of the 2017 World Workshop of Periodontology (WWP) specified the bone loss levels of more than 3mm in addition to presence of bleeding on probing (Berglundh et al., 2017). In a subsequent publication, the 2017 WWP criteria demonstrated a high specificity but low sensitivity using the aforementioned approach and found the combination of BOP and bone loss of more than 2mm to be more accurate (Romandini et al., 2021). It is thought that peri-implantitis is the result of submucosal polymicrobial biofilm formation that causes breakage of the mucosal seal to the implant (Schwarz et al., 2018; Kensara et al., 2021). Peri-implantitis is a concern for clinicians and patients because progressive bone loss can lead to the loss of the implant (Caton et al, 2018).

1.7 *Supportive Care & Existing Treatments*

Maintenance and supportive therapy with regular recall intervals tailored to patients' specific needs has been proven to lower the biological complications (Renvert et al., 2015; Monje et al., 2016). A 5-year follow-up study on patients with pre-existing peri-implant mucositis observed that individuals without preventive maintenance exhibited a significantly higher incidence of peri-implantitis (44%) compared to those receiving

supportive care (18%) (Costa et al., 2012). At the final examination, inadequate plaque control emerged as the most robust statistical predictor for peri-implantitis with high Odds Ratios (ORs) (Ferreira et al., 2006; Aguirre-Zorzano et al., 2015; Rokn et al., 2017)

Peri-implantitis can be prevented by supportive care if the patient is compliant with home care and maintenance where evaluation is done at the patient and implant levels (Wilson Jr et al., 2014). At least 2 visits per year are recommended and are associated with prevention of peri-implantitis in healthy patients (Monje et al., 2017). There is no definitive treatment of peri-implantitis, with articles publishing empirical strategies. Non-surgical debridement and surgical techniques to degranulate bony defects and decontaminate the implants have been described. Regenerative techniques use varying techniques, materials, small patient sample size, and varying lesion configuration. The Third European Association for Osseointegration (EAO) Consensus Conference on Peri-implant tissue destruction describes three adjunctive non-surgical techniques for implant decontamination: submucosal air-polishing, erbium-doped yttrium-aluminum-garnet (ER:YAG) laser treatment, and locally delivered antimicrobials. In regards to treatment with ER:YAG laser, Clem et al identified twenty patients with 23 implants and 84 defects (Clem et al., 2019). They show reduced probing depths at 12 months (Clem et al., 2019). The issue with this study is the very small sample size, the lack of identification of lesion configuration, and the short follow-up. Moreover, several studies have demonstrated no advantage of laser therapy to conventional non-surgical treatment, nor statistically significant additional benefit beyond what surgical treatment alone provides (Faggion et al., 2019; Mills et al., 2018). Submucosal air polishing avoids damaging the titanium implant surface but produces the risk of emphysema (Basseti et al., 2014). Regarding

locally delivered antibiotics, Winkelhoff in 2012 reviewed the literature on the use of systemic and locally delivered antibiotics in treatment of periodontitis (Winkelhoff, 2012). Two studies on systemically administered antibiotics lacked controls, and the studies that looked at locally administered antibiotics were used in conjunction with mechanical debridement and locally delivered antimicrobials, such as chlorohexidine or hydrogen peroxide, and the implants treated had deep pockets and advanced bone loss. Yet there was no control group in both studies (Winkelhoff, 2012).

1.8 *Risk Factors for Peri-Implantitis*

Risk factors for peri-implantitis include history of chronic or aggressive periodontitis, poor oral hygiene, lack of regular maintenance therapy, smoking, diabetes, systemic inflammatory disease, cardiovascular disease, bone-metabolism disease, implant position, prosthetic design, excess cement, insufficient keratinized gingiva, and titanium particles. Strong evidence exists for the correlation between peri-implantitis and a history of periodontitis, poor oral hygiene, and a lack of regular maintenance therapy (Schwarz et al, 2018). Lee et al found that a lengthier follow-up duration is linked to an increased prevalence of peri-implantitis (Lee et al., 2017). Severe periodontitis ranks 6th in the most chronic prevalent disorders with 68% of persons ≥ 65 years affected with chronic periodontitis in the USA (Eke et al., 2015). Several studies have shown that patients with a history of periodontitis had significantly higher odds for peri-implantitis when compared to counterparts (Roos-Jansaker et al., 2006 a,b,c; Koldsland et al., 2010; Koldsland et al., 2011; Karoussis et al. 2003, Hammerle & Glauser, 2004, Schou et al. 2006, Ferreira et al. 2006, Simonis et al. 2010, De Boever et al. 2009).

There is an association of prosthetic features, such as over-contoured prosthesis, and emergence angle greater than 30 degrees, a convex emergence profile, that predispose implants to peri-implantitis (Yi et al., 2020). Katafuchi et al found that in bone-level implants, restorations with a combined convex profile and an emergence angle of >30 degrees had the highest rate of peri-implantitis (Katafuchi et al., 2017). Katafuchi et al did not observe this association in tissue-level implants (Katafuchi et al., 2017). Serino et al. found that areas where restoration is inaccessible for hygiene to be more associated with per-implantitis (65%) than the cleansable sites (18%) (Serino et al., 2016). The deeper the position of the margin and larger the diameter of the implant, the greater the amount of undetected cement (Wilson et al., 2009; Staubli et al., 2016). Dental radiographs are not a reliable method to detect excess cement due to the radiolucency of methacrylate (Wilson et al., 2009; Linkevicius et al., 2013). A study found radiopaque particles in 34 out of 36 biopsies, predominantly titanium and dental cement surrounded by inflammatory cells (Wilson et al., 2015). Jepsen et al categorized the presence of excess cement as a local risk indicator for peri-implant mucositis, since biofilm adherence is intensified on the rough surface of the cement (Jepsen et al., 2015). The observed variations in results can be attributed to discrepancies in the classification of smokers and non-smokers, where the criteria employed to categorize individuals as "smokers" differ notably among various studies. Additionally, the reliance on patient-reported data for assessing smoking status further contributes to these dissimilarities (Schwarz et al., 2018). Smoking has a chronic suppressive effect on vasculature (Palmer et al., 2005). Smokers have decreased bleeding on probing (Bergstrom & Bostrom., 2001). Smoking negatively impacts innate and adaptive immune responses (Palmer et al., 2005). Smoking increases the number of

neutrophils found in the systemic circulation (van Eeden & Hogg 2000, Iho et al. 2003, Sorensen et al. 2004) but transmigration across the gingival sulcus is impaired in tobacco smokers (Eichel & Shahrik 1969, Pauletto et al. 2000). Smokers have a reduced CD4/CD8 ratio (Wallace et al. 1994). On smoking cessation, CD4 cells count returns to normal levels (Ginns et al. 1982, Loos et al. 2004). Smoking affects fibroblast function, recruitment, and adhesion to root surface (Gamal and Bayomy (2002). Nicotine and P. gingivalis lipopolysaccharide (LPS) can synergistically upregulate IL-6, IL-7, IL-8, IL-10, IL-15, and interferon-gamma (IFN-gamma) production (Wendell and Stein, 2001; Almasri et al., 2007). In a 10-year prospective cohort study evaluating 112 implants, 21 implants in 8 patients who lost their teeth due to chronic periodontitis, with 10 implants placed in smokers, and 91 implants in 45 patients without a history of periodontitis, with 19.78% of implants (18 implants) placed in smokers (Karoussis et al., 2003). In patients with a history of periodontitis, the survival rate of implants was 80% in smokers and 100% in nonsmokers, demonstrating that smoking patients susceptible to periodontitis are associated with a substantiated higher risk of implant loss compared to both non-smoking patients with periodontitis and those without periodontitis (Karoussis et al., 2003). Additionally, patients with a history of periodontitis exhibited lower implant survival rates (90.5% vs. 96.5%), significantly higher complication rates (28.6% vs. 5.8%), and significantly lower success rates (e.g., 71.4% vs. 94.5%) than patients who lost their teeth due to reasons unrelated to periodontitis, such as caries and fractures (Karoussis et al., 2003). Specifically, 18% of implants in smokers developed peri-implantitis, compared to only 6% of non-smoking counterparts (Karoussis et al., 2003).

Diabetes mellitus encompasses a cluster of metabolic disorders characterized by hyperglycemia, with type 1 involving autoimmune destruction of insulin-producing β-cells and type 2 marked by insulin resistance (Atkinson et al., 2014). The diagnostic criteria for diabetes mellitus include the following indicators: fasting plasma glucose level in venous plasma of ≥126 mg/dL, HbA1c level of ≥6.5%, 2-hour post-load plasma glucose measurement of ≥200 mg/dL, or a random plasma glucose level of ≥200 mg/dL when hyperglycemia symptoms polydipsia, polyphagia, or polyuria are present (Atkinson et al., 2014). Alberti et al. conducted a study on 204 subjects who received 929 implants (Alberti et al., 2020). Among them, 23 patients developed peri-implantitis, an overall prevalence of 11.3% (Alberti et al., 2020). The patient-level cumulative implant survival rate was 95.42% after 10 years of surgery, with no significant difference between diabetic and non-diabetic patients (96.51% and 94.74%, respectively). Notably, glycemia levels and HbA1c were not recorded during follow-up visits, and only one patient had uncontrolled diabetes in the study (Alberti et al., 2020). Other studies found that increasing HbA1c a significant deterioration in the clinical indicators for peri-implantitis could be observed (Al-Sowygh et al., 2018; Eskow et al., 2017).

1.9 *Matrix metalloproteinases (MMPs)*

Matrix metalloproteinases (MMPs) are capable of extracellular matrix (ECM) breakdown, contributing to tissue remodeling. MMPs are regulated by the tissue inhibitors of metalloproteinases (TIMPs). Imbalance of MMPs/TIMPs is seen in cancer and periodontitis (Verstappen and Von den Hoff, 2006) The combination of nicotine and *P. gingivalis* and LPS has a synergistic effect on collagen degradation (Zhou et al., 2007; Katono et al., 2009; Zhang et al., 2010; Kim et al., 2012).. Zhou et al demonstrated

that cigarette smoke condensate (CSC) destroyed the balance between the MMPs and TIMPs at the transcriptional and translational levels, and increased the rate of MMP activation (Zhou et al., 2007). Katono et al found decreased alkaline phosphatase (ALPase) in osteoblast cells cultured with nicotine and LPS (Katono et al., 2009). This demonstrates the severity of periodontitis in smokers compared to non-smokers. Cigarette smoking negatively impacts periodontal treatment (Johannsen etl al., 2014; Trombelli Let al., 2018). Smoking increases oxidative stress and collagen metabolism (Geisinger et al., 2017). Smoking increases the risk of developing peri-implantitis by two-fold [Dreyer et al., 2018]. Smoking and a history of periodontitis are each associated with increased severity of peri-implantitis evident by peri-implant marginal bone loss (Saaby et al., 2016).

1.10 *Etiology of Peri-Implantitis*

Contemporary endosseous dental implants consist of a bone-level or tissue-level body, and a prosthetic abutment that is connected to the implant using a screw. A microgap that ranges between 0.1 and 10 μm in size is thus formed at the implant/abutment interface (IAI). In the oral cavity, bacterial leakage into the IAI is facilitated by the microgap and the micromotion during mechanical loading at the IAI (Liu et al, 2017; Tallarico et al, 2017; Cosyn et al, 2011). The composition and surface characteristics of the implants and prosthetic abutment are important considerations in peri-implantitis (do Nascimento et al, 2014). They may impact the adhesion and maintenance of oral biofilm and, consequently, aid or inhibit the colonization of microbes (Teughels et al.2006). Bacterial passage along this interface has been documented by in-vitro and in-vivo studies (Steinebrunner et al.2005; do Nascimento et al. 2012) The internal surface of loaded dental implants presents a unique environment for bacteria

(Tallarico et al, 2017; Cosyn et al, 2011). In a controlled cross-sectional study, Kensara et al characterized the microbiome composition within dental implants in healthy patients and patients with peri-implantitis using next-generation sequencing of 16S rRNA gene (Kensara et al, 2023). There was a significant difference in the microbiome composition inside healthy implants and implants with peri-implantitis (Kensara et al, 2023). The findings of this investigation showed that Prevotella, Streptococcus, Parvimonas, Fusobacterium, Veillonella, and Slackia were in high abundance and prevalence within healthy implants (Kensara et al, 2023). These species may mediate the symbiotic relationship within the healthy microbiome and can serve as biomarkers for periimplant health (Kensara et al, 2023). Motile microbes such as E. casseliflavusis could move across the IAI (Kensara et al, 2023). Peri-implantitis exhibited higher levels of abundance and prevalence of facultative anaerobic Gram-positive lactic acid bacteria (LAB) Enterococci, especially E. casseliflavus (Kensara et al, 2023). There was a reduction in the bacterial diversity of peri-implantitis, supporting the suggestion that higher diversity indicates a healthy microbiome (Lloyd-Price et al, 2016, Kensara et al, 2023). This phenomenon could result in dysbiotic bacteria evading the immune system.

Studies suggest that peri-implantitis is a polymicrobial infection that is different in composition to periodontitis (Dabdoub et al., 2013; Lafaurie et al., 2017). Inflammation is more aggressive, is beyond the junctional epithelium, and is closer to bone than in periodontitis (Berglundh et al., 2004). A recent systematic review analyzed forty articles, twenty of which compared the microbiological profile of peri-implantitis with healthy implants (Kensara et al, 2021). The study found that the polymicrobial biofilm is composed of obligate anaerobe Gram-negative bacteria (OAGNB), such as *Tannerella forsythia* [Tf],

Treponema denticola [Td], *Prevotella intermedia* [Pi], *Porphyromonas gingivalis* [Pg], *Fusobacterium nucleatum* [Fn], *Centipeda periodontii* [Cp], and *asaccharolytic anaerobic Gram-positive rods* (AAGPRs) such as *Eubacterium nodatum* [En], *Eubacterium brachy* [Eb], *Slackia exigua* [Se], *Filifactoralocis* [Fa], *Parascardovia denticolens* [Pd], and *Parvimonasmicrap* [Pm] (Kensara et al, 2021). This biofilm and resulting inflammation is associated with the upregulation of several pro-inflammatory mediators, such as IL-1β, IL-6, IL-17, and TNF-α and osteolytic mediators, such as RANK, RANKL, Wnt5a, proteinase enzymes, MMP-2, MMP-9, and Cathepsin-K (Kensara et al, 2021).

Epstein-Barr virus (EBV) and Human cytomegalovirus (HCMV) have been found in the plaque of peri-implantitis sites (Jankovic et al 2011; Jankovic et al 2011). Co-infection by HCMV and EBV was recorded in 33.3% of the peri-implantitis sites and 0% of the healthy sites (Jankovic, 2011).

Peri-implantitis develops due to the imbalance between microbial invasion to the dental implant and host's immune response. The invasion of pathogens triggers abnormal immunoinflammatory mediator expression leading to osteolytic reactions (Kensara et al 2021). Proinflammatory cytokines such as IL-1β, IL-17, IL-6, and TNFα, are upregulated in peri-implantitis patients (Faot et al, 2015; Rakic et al, 2014). Bone remolding mediators such as RANKL, and proteolytic enzymes such as MMP-2, MMP-9, and cathepsin-K are also upregulated in peri-implantitis patients (Arıkan et al, 2011; Yakar et al, 2019; Che et al, 2017). These studies suggest that the upregulated mediators be used as a potential drug target for Peri-Implantitis (Che et al, 2017; Yakar et al, 2019; Arıkan et al, 2011; Rakic et al, 2014). An ideal biomarker exhibits high sensitivity, specificity, and positive or negative predictive values (Yakar et al, 2019). There is a lack of optimized protocols providing high

levels of detection (Rakic et al, 2014). The inconsistency observed in cytokines, enzymes, and proteases investigated in the literature to be used as potential biochemical markers is likely attributed to the unique nature of peri-implant crevicular fluid (PICF) as a diagnostic medium in the majority of the studies (Rakic et al, 2014). PICF stands out as an oral fluid with notably low protein content, posing a challenge for commercial diagnostic assays designed primarily for media with considerably higher protein content like serum (Rakic et al, 2014). Moreover, PICF exhibits relatively limited flow compared to other media such as serum, which introduces additional challenges in standardizing detection levels (Rakic et al, 2014). It would make sufficient sample collection of healthy subjects a challenge. Consequently, various studies present data in percentages of detection or concentrations, contributing to a lack of consistency in reported results in the literature (Rakic et al, 2014). This inconsistency complicates the interpretation of the diagnostic significance of these biomarkers (Rakic et al, 2014).

However, there are numerous unique mediators that might have a potential role in peri-implantitis progression that were never studied. I will investigate a new mediator, IL-18. Peri-implantitis is a multi-factorial disease that is still poorly understood. The goal of this study was to provide information to aid in our understanding of peri-implantitis. Faot et al suggested that inflammatory mediators present in peri-implant crevicular fluid samples are potential diagnostic markers aiding in the early detection of Peri-Implantitis (Faot et al, 2015). Faot et al also suggested that the immunoinflammatory processes leading to tissue degradation are complex and may not consistently manifest in a single instance of fluid collection (Faot et al, 2015). The intricate cytokine network, activated within diseased

peri-implant tissue, exhibits complexity due to the overlapping roles of numerous cytokines and is susceptible to fluctuations based on the activity of the disease (Faot et al, 2015).

1.11 *IL-18*

IL-18 is a member of the TH1 family of cytokines and is secreted by macrophages and monocytes (Dinarello, 1999). The mature form of IL-18 is produced by the action of the intracellular cysteine protease called IL-1β–converting enzyme (ICE) (Dinarello). ICE cleaves (proIL-18), the inactive precursor form that lacks a signal peptide (Dinarello, Johnson et al, 2005). IL-18 plays a role in the progression of inflammation as it has chemotactic, proinflammatory, and proangiogenic functions (Johnson et al, 2005; Puren et al., 1998). IL-18 induces human CD4+ lymphocyte chemotaxis as well as polarization and migration of CD4+ and $CD8^{+}$ mononuclear cells (Komai-Koma et al., 2003). IL-18 stimulates the production of angiogenic TNF-α and induces endothelial cell chemotaxis (Park et al., 2001). The disc angiogenesis system allows reproducible testing of angiogenic antagonists and agonists and provides histological data and quantitative measurements of vessels' growth (Fajardo et al., 1988). IL-18 at 10 nM induced angiogenesis at a 4-fold increase in the amount of hemoglobin compared to control (Park et al., 2001).

IL-18 increases concentrations of cell adhesion molecules and chemokines, inflammatory mediators (such as nitric oxide) and the rates of neutrophil activation. IL-18 has been linked to the development of inflammatory diseases and immune activation, such as sepsis (Grobmyer et al. 2000; Netea et al. 2000), rheumatoid arthritis (Yamamura et al. 2001), atherosclerosis (Mallat et al. 2001a), Wegener's granulomatosis and systemic lupus erythematosus (Novick et al 2009; Novick et al 2011). IL-18 is upregulated in periodontitis patients (Orozco et al, 2006; Johnson et al, 2005; Figueredo et al., 2008).

IL-18 induces the release of matrix metalloproteinase-9 (MMP)-9 that mediates periodontal tissue destruction and degradation of the basement membrane and extracellular matrix components (Buduneli et al, 2011; Nold et al, 2003). MMP-9 is elevated in periodontal disease, and it is suggested to be a useful biomarker for periodontal disease progression (Nold et al., 2003; Ramseier et al, 2009). MMP-9 is also elevated in peri-implantitis patients (Zhang et al., 2020). The interaction between IL-18 and MMP-9 demonstrates a role of IL-18 in the host response and pathogenesis of peri-implantitis and periodontal disease. It is the exaggerated host response and faulty inflammation that contributes to the severity of the destruction. IL-18 increased the number of osteoclasts and bone resorption area on dentine slices in the coculture of human osteoclast precursors with T cells (Dai et al., 2004).

1.12 *IL-18BP*

The biological activity of IL-18 is downregulated by the antagonist IL-18 binding protein (IL-18BP), which binds to IL-18, thus inhibiting its interaction with the IL-18 receptor (Novick et al, 1999). There are four isotypes of human IL-18BP (IL-18BPa-d), with IL-18BPa having the greatest affinity for IL-18 (Novick et al, 2011). It antagonizes IL-18 by forming a high affinity complex, thereby suppressing the production of IFN-γ, resulting in reduced T-helper type 1 immune responses (Novick et al, 1999). IL-18BPa is secreted by human monocytes (Kim et al, 2000). IL-18BPa is constitutively present in human serum and is significantly elevated in individuals with sepsis (Novick et al, 2001).

Porphyromonas gingivalis (Pg) Lipopolysaccharide (LPS) stimulated IL-18 secretion in cultures of the human monocytic cell line THP-1 (Hamedi et al, 2009). Further, another study found that LPS of *P. gingivalis* (Pg) caused simultaneous secretion of IL-

18BPa (Foster et al, 2007). Moreover, a study demonstrated increased levels of IL-18 after injection of *P. gingivalis (Pg)*-LPS into rodents' gingival sulcus (Li et al., 2021). This demonstrates an interaction between IL-18 and IL-18BPa and the microbiota associated with peri-implantitis and periodontal disease.

There are no research studies on peri-implantitis that evaluate the level of IL-18. IL-18 is highly expressed in periodontitis patients and stimulated by LPS of periopathogenic bacteria. A previous study of pooled saliva samples in our lab found that IL-18BP was highly expressed in pooled saliva of peri-implantitis subjects (Kensara, 2023).

The study significance is in that IL-18 if proven to have a role in peri-implantitis can be used as a biomarker for diagnostic aid, to monitor the severity of disease, or it can be a potential therapeutic target via pharmacological agents or immunomodulatory strategies. It can also open the door to tailoring the treatment based on individual's cytokine profile. Current studies are ongoing in targeting IL-18 cytokine by using anti IL-18 antibodies, inhibiting secretion from cells, and using IL-18BPa or IL-18 species in inflammatory diseases such as Rheumatoid arthritis (RA) (Gracie et al, 2004). Clinical trials are undergoing in using IL-18BP to block IL-18 cytokines to treat inflammatory disease (Dinarello et al, 2013). IL-18BP has been used to reduce some colitis induced by antigen sensitization (Hove et al, 2001).

1.13 *External Implant Surface & Treatments*

Over the last 20-25 years, implant surface topography has been studied and modified through a variety of methods to increase surface roughness, bone to implant contact (BIC), surface chemistry and biocompatibility, and cleanliness, in order to enhance

healing, increase the rate of bone apposition, and decrease the osseointegration time, allowing clinicians to immediately/early place and load dental implants (Aljateeli et al., 2013). Rough surfaces have shown torque to failure values higher than implants with smooth machined profiles (Aljateeli et al., 2013). These surface modifications include mechanical and chemical treatments that are classified into additive and subtractive processes (Aljateeli et al., 2013). Additive processes include titanium plasma spray, hydroxyapatite coatings & calcium phosphate, oxidation, and ion deposition. Subtractive processes include grit-blasting, acid-etching, electropolishing, mechanical polishing, and laser microtexturing (Aljateeli et al., 2013). Biological coatings include growth factors such as Bone morphogenetic proteins (BMPs), transforming growth factor-b1 (TGF-b1), platelet-derived growth factor, insulin-like growth factors 1 and 2, and peptides (Aljateeli et al., 2013).

Chapter 2 Aim and Null Hypothesis

This study will add to our understanding of peri-implantitis and help discover a new therapeutic direction.

Aim: To evaluate the concentration level of IL-18 in saliva samples using high-sensitivity enzyme-linked immunoabsorbent assays (ELISA).

Null Hypothesis: There is no difference in salivary levels of IL-18 between subjects with healthy dental implants and subjects with dental implants with peri-implantitis.

Chapter 3 Materials and Methods

3.1 *Patient Population*

Institutional Review Board (IRB) at the University of Maryland, Baltimore for all aspects of study protocol was obtained (HB #00073350). Medical history was obtained and documented. The study will be explained to patients and informed consent was obtained. Saliva was collected from 10 healthy and 14 peri-implantitis adult patients in the graduate Prosthodontics program at the University of Maryland. There are 12 male and 12 female subjects.

Inclusion criteria includes healthy adult patients who are not diagnosed with systemic inflammatory diseases, diabetes, endocarditis, or autoimmune disease, bone related disease, history of radiation or cancer, or other significant medical history. Patients have either a healthy dental implant, or an implant diagnosed with peri-implantitis, which has been in function for > 12 months. Patients excluded are smokers, patients who have significant medical history (systemic inflammatory diseases, diabetes, endocarditis, autoimmune disease, bone related disease, history of radiation or cancer, or other significant medical history), patients who have taken antibiotics or corticosteroids treatment in the past 3 months, patients with periodontitis, pregnant or nursing women, and patients who present with uncooperative behavior. An experienced periodontist established the peri-implantitis/healthy-implant diagnoses based on the current definition of peri-implantitis by the AAP following a thorough clinical and radiographic examination.

3.2 *Saliva Collection*

Unstimulated saliva was collected from participants. Patients were seated on the dental chair in a relaxed position and asked to depress their chin and pool saliva in the bottom of their mouths. Saliva was collected from participant subjects into 15 ml tubes every minute for 5 minutes. Collected saliva was then centrifuged for 5 minutes at 10,000 x g. The aliquot layer was collected and immediately stored at -80°C until analysis.

3.3 *Cytokine Detection*

Sandwich enzyme-linked immunosorbent assay (ELISA) kit (Quantikine HS, R&D Systems, Minneapolis, MN, USA) was used to measure the levels of IL-18 in saliva. Aliquots from saliva was added in triplicate to the wells of microtiter plates for determination of IL-18. All assays using commercial kits (Quantikine HS, R&D Systems, Minneapolis, MN, USA) were performed according to the manufacturers' instructions. Results were expressed as picograms of cytokine per milligram of periodontal tissue (pg/mg). The optical density of each well was determined using a microplate reader (CytationTM 5, BioTek® Instruments, Winooski, Vermont, USA) set to 450 nm. Wavelength correction was set to 540 nm. This corrected for optical imperfections in the plate.

3.4 *Sample Preparation*

Saliva samples were diluted 2-fold dilution due to a matrix effect. Matrix effect (ME) is ion inhibition or enhancement due to the presence of a matrix or other interferences in the sample (Marchi et al., 2010). ME is responsible for poor and unreliable data in a

quantitative assay, which can negatively affect the reproducibility, linearity, and accuracy of the method and lead to erroneous quantitation (Marchi et al., 2010).

3.5 *Reagent Preparation*

Table 1: Reagent Preparation and resultant product used in the study.

Reagent Preparation	Product
Wash Buffer Concentrate 20mL	Wash Buffer 500 mL
Color Reagents A and B	Substrate Solution
Calibrator Diluent RD5P	Calibrator Diluent RD5P (diluted 1:5) 50 mL
Human Total IL-18 standard	Stock solution of 10,000 pg/mL

All reagents were brought to room temperature before use.

Wash Buffer - 20 mL of Wash Buffer Concentrate was added to 480 mL of distilled water to prepare 500 mL of Wash Buffer (Table 3-1).

Substrate Solution - Color Reagents A and B were mixed in equal volumes within 15 minutes of use. They were also protected from light. 200 µL of the resultant mixture is required per well (Table 3-1).

Calibrator Diluent RD5P (diluted 1:5) - 10 mL of Calibrator Diluent RD5P was added to 40 mL of deionized or distilled water to prepare 50 mL of Calibrator Diluent RD5P diluted 1:5 (Table 3-1).

Human Total IL-18 standard was reconstituted with distilled water. This reconstitution produces a stock solution of 10,000 pg/mL (Table 3-1). The standard was mixed to ensure complete reconstitution. The standard was allowed to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Four hundred and fifty µL of Calibrator Diluent

RD5P (diluted 1:5) was pipetted into the 1000 pg/mL tube. Two hundred µL was pipetted into the remaining tubes. The stock solution was used to produce a dilution series. Each tube was mixed thoroughly before the next transfer. The 1000 pg/mL standard serves as the high standard. Calibrator Diluent RD5P (diluted 1:5) serves as the zero standard (0 pg/mL).

3.6 *Assay Procedure*

In this study, the Quantikine™ Human Total IL-18/IL-1F4 Immunoassay was conducted using a 96-well microplate format. First, 50 µL of Assay Diluent RD1N was added to each well. Subsequently, 50 µL of standard, control, or sample was added to their respective wells and covered with adhesive strips. The plate was incubated for 2 hours at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at 500 ±50 rpm. A plate layout was used to record standards and samples assayed.

Following incubation, each well underwent a series of washes, with four washes performed in total. For each wash, the wells were filled with 400 µL of Wash Buffer and then completely aspirated, repeating this process three times. After the last wash, any remaining Wash Buffer was removed, and the plate was inverted and blotted against clean paper towels.

Next, 200 µL of Human Total IL-18 Conjugate was added to each well, followed by another incubation for 1 hour at room temperature on the shaker. The aspiration/wash procedure was repeated.

Subsequently, 200 µL of Streptavidin-HRP was added to each well and incubated for 30 minutes at room temperature on the shaker. The aspiration/wash process was repeated once more.

Then, 200 μL of Substrate Solution was added to each well, and the plate was protected from light using aluminum foil. Incubation was carried out for 30 minutes at room temperature on the benchtop.

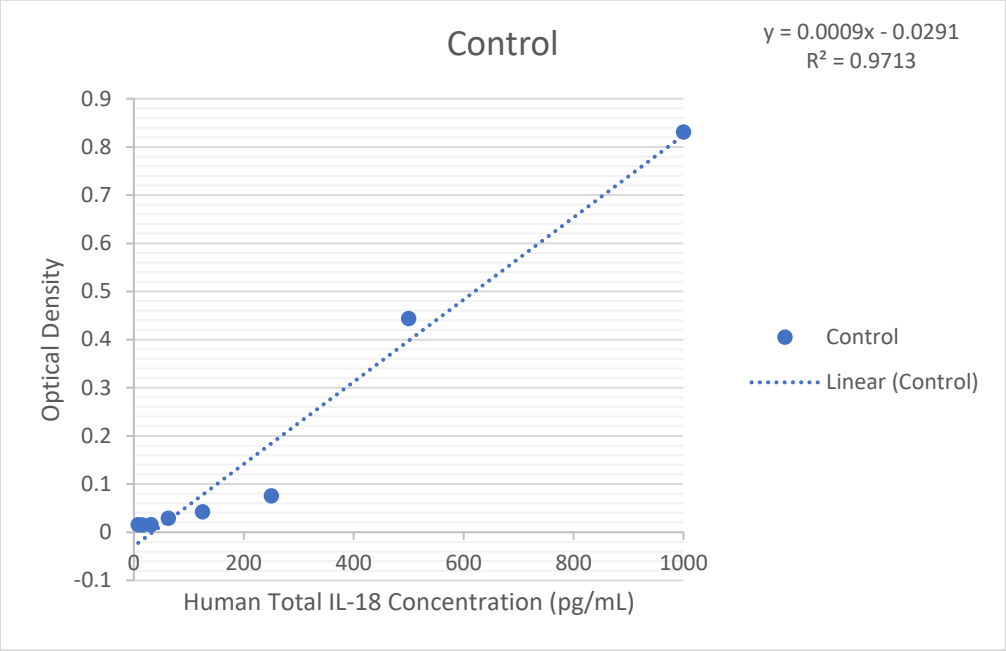
To stop the reaction, 50 μL of Stop Solution was added to each well, resulting in a change of color from blue to yellow.

Within 30 minutes of stopping the reaction, the optical density of each well was determined using a microplate reader (CytationTM 5, BioTek® Instruments, Winooski, Vermont, USA) set to 450 nm. Wavelength correction was set to 540 nm or 570 nm, as appropriate. This allowed for the quantification of the Human Total IL-18 levels in the samples assessed.

3.7 *Statistical Analysis*

The duplicate readings for each standard, control, and sample were averaged and then the average zero standard optical density (O.D.) was subtracted. A standard curve was created by plotting the mean absorbance for each standard on the y-axis against the human Total IL-18 concentration on the x-axis (Figure 1). Best fit curve was established through the points on the graph (Figure 1). The concentration read from the standard curve was multiplied by the 2x (dilution factor).

Figure 1 Saliva assay of control standard curve
Standard curve of Saliva Assay Human Total IL-18 Controls (R&D Systems®, Catalog # QC260) plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis.



Data from triplicate experiments are expressed as means. All statistical analyses were performed using Microsoft® Excel® for Microsoft 365 MSO (Version 2308 Build 16.0.16731.20182) 64-bit. Statistical comparisons were tested for normality. The calculated concentration median was obtained for the two groups, followed by the Mann Whitney U test ($P<0.05$).

$$U_1 = n_1 n_2 + \frac{n_1(n_1+1)}{2} - R_1$$

$$U_2 = n_1 n_2 + \frac{n_2(n_2+1)}{2} - R_2$$

Where R1 represents the summation of ranks within the healthy group, and R2 represents the summation of ranks within the peri-implantitis group.

Chapter 4 Results:

A total of 25 individuals met the eligibility criteria, and consented to participate, and were enrolled as subjects. Patients with both healthy implants and implants suffering from peri-implantitis were excluded. Following initial recruitment, one participant opted to withdraw from the study post-sample collection. Consequently, the final cohort consisted of 24 subjects, comprising 12 males and 12 females, with an average age of 63.53 ±15.85 years standard deviation. The demographic and subject-specific characteristics are detailed in Tables 1 and 2, encompassing both the study participants and the implants under scrutiny.

Within this cohort of 24 subjects, an investigation was conducted on a total of 33 implants. Among these implants, 22 were diagnosed with peri-implantitis, while the remaining 11 were designated as healthy controls, as delineated in Table 2.

Table 2 Demographic data of subjects included in the study

	Subjects with healthy implants	Subjects with peri-implantitis
*Age (*years*)*	70.6 ±16.33	60 ±16.43
Gender (Males:Female)	4:2	6:7

Table 3 Characteristics of dental implants included in the study

	Healthy implants	Peri-implantitis
Implant manufacturer:		
NobelReplace® ;Replace Select ™	4	9
BioHorizons®	2	1
CAMLOG®	1	-
Biomet 3i ™	4	6
Astra Tech®	-	1
SPI implant	-	2
Unknown	-	3
Implant location:		
Maxillary anterior	6	6
Maxillary posterior	-	6
Mandibular anterior	-	1
Mandibular posterior	5	9
Platform switch (yes:no)	2:9	12:7
Unk*nown*	-	3
Average years in function	5.13 ±4.79	9.61 ±8.79

Table 4 Median values of IL-18 analytes expressed in pg/ml, and statistical analysis:

	Healthy implant	**Peri-implantitis**	**Mann Whitney U test**
IL-18	2.23 pg/ml	1.92 pg/ml	*P = 0.16* Insignificant

The Mann Whitney U test demonstrated that there were no statistically significant differences in individual salivary concentration of IL-18 among subjects with peri-implantitis and healthy control (*P = 0.16*, Table 3).

Figure 2 Boxplot highlighting median concentration of IL-18 in saliva (Pg/ml) by ELISA and and 25-75 percentiles.

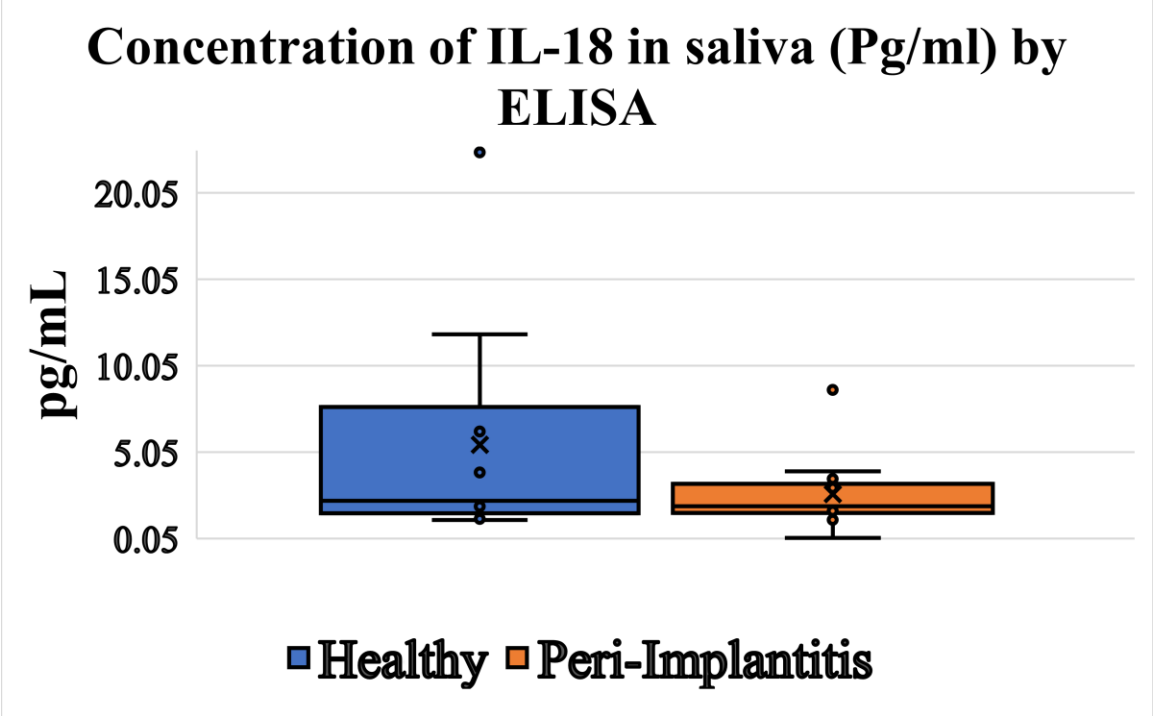


Table 5 Mean, Range, and Standard Deviation values of IL-18 analytes expressed in pg/ml:

IL-18	Healthy implant	Peri-implantitis
Mean	5.47 pg/ml	2.61 pg/ml
Range	21.26 pg/ml min 1.13 pg/ml max 22.39 pg/ml	8.58 pg/ml min 0.079 pg/ml max 8.66 pg/ml
Standard deviation	6.80 pg/ml	2.04 pg/ml

The Median values of IL-18 analytes were 1.92 pg/ml for peri-implantitis and 2.23 pg/ml for healthy control (Figure 2, Table 3). The range was 8.58 pg/ml for peri-implantitis [min 0.079 pg/ml – max 8.66 pg/ml] (Figure 2, Table 4). The range was 21.26 pg/ml for healthy control [min 1.13 pg/ml – max 22.39 pg/ml] (Figure 2, Table 4). The mean was 5.47 pg/ml for healthy control and 2.61 pg/ml for peri-implantitis (Figure 2, Table 4). The standard deviation was 6.80 pg/ml for healthy control and 2.04 pg/ml for peri-implantitis (Table 4).

Chapter 5 Discussion and Conclusion

This is the first study using quantitative sandwich enzyme immunoassay technique to specifically evaluate salivary IL-18 levels in healthy patients and patients with peri-implantitis. The Quantikine™ Human Total IL-18/IL-1F4 Immunoassay is a 4.0 hours solid-phase ELISA designed to measure human IL-18 in saliva (R&D Systems, 2021). This assay demonstrates high sensitivity and accuracy in recognizing and quantitating natural and recombinant human Total IL-18 (R&D Systems, 2021). Notably, no significant cross-reactivity or interference was observed (R&D Systems, 2021). In the evaluation of fifty-two assays, the minimum detectable dose (MDD) of human Total IL-18 ranged from 0.296 to 5.15 pg/mL, with a mean MDD of 1.25 pg/mL for the RD1N saliva assay (R&D Systems, 2021). The MDD was determined using a calculation based on the mean optical density (O.D.) value of twenty zero standard replicates plus two standard deviations to derive the corresponding concentration (R&D Systems, 2021). The immunoassay is calibrated against a highly purified E. coli-expressed recombinant human IL-18 produced by R&D Systems (R&D Systems, 2021). Employing a quantitative sandwich enzyme immunoassay technique, the assay involves pre-coating a microplate with a monoclonal antibody specific for human Total IL-18 (R&D Systems, 2021). Standards and samples are then added to the wells, and any IL-18 present binds to the immobilized antibody (R&D Systems, 2021). Subsequent steps involve the addition of a biotinylated monoclonal antibody specific for Total IL-18, followed by an enzyme-linked streptavidin, and a substrate solution to generate color proportionate to the initial IL-18 binding (R&D Systems, 2021). Finally, the color development is halted, and the intensity of the color is measured (R&D Systems, 2021).

IL-18 is upregulated in periodontitis patients (Orozco et al, 2006; Johnson et al, 2005; Figueredo et al). In a previous study in our lab that compared 105 salivary inflammatory mediators between subjects with peri-implantitis and those with healthy implants using an antibody array, the level of IL-18BPa was elevated 14 folds, in peri-implantitis subjects compared to healthy subjects using the proteome assay (Kensara, 2023). However, when IL-18 was individually tested, ELISA failed to find any difference between the healthy and peri-implantitis groups. The null hypothesis was accepted.

Our study found no difference between IL-18 levels in the saliva of healthy patients and peri-implantitis patients. The Median values of IL-18 analytes were 1.92 pg/ml for peri-implantitis and 2.23 pg/ml for healthy control (Table 3). The range was 8.58 pg/ml for peri-implantitis [min 0.079 pg/ml – max 8.66 pg/ml] (Table 4). The range was 21.26 pg/ml for healthy control [min 1.13 pg/ml – max 22.39 pg/ml] (Table 4). The mean was 5.47 pg/ml for healthy control and 2.61 pg/ml for peri-implantitis (Table 4). The standard deviation was 6.80 pg/ml for healthy control and 2.04 pg/ml for peri-implantitis (Table 4).

Previous research in our lab found IL-18BPa levels to be high using human antibiotic cytokine assays (Kensara et al., 2023). The high expression of IL18BPa in the results could be multifactorial. However, in these experiments, saliva was pooled which may have masked inter-individual variability or data could have been skewed by few abnormal samples; IL18 may have been highly expressed in one or a few sites or one or a few patients. A second explanation is subject variability due to microbiome difference and diversity, causing varying expression of IL18. A third explanation is that IL-18 may be highly expressed in individuals with systemic inflammation who were excluded in our

study. There are factors, such as smoking, diabetes, antibiotics use, and anti-inflammatory medications, that can cause a shift in the microbiome composition and the inflammatory response of the host (Tsigarida et al, 2015; Ganesan et al, 2017). A fourth explanation is that there is simply no difference, and IL-18 is not highly expressed in the saliva of patients diagnosed with peri-implantitis. Our study aims to add in our understanding of peri-implantitis by measurement of salivary expression of IL-18 in peri-implantitis and healthy patients. IL-18 is one of the cytokines possibly involved in a complex biochemical network. If IL-18 is not highly expressed in peri-implantitis, results could be interpreted that periodontitis and peri-implantitis develop through different mechanisms. It can also suggest that peri-implantitis may require a different diagnostic or therapeutic direction.

Peri-implantitis is a multifactorial disease that has complex underlying mechanisms related to biofilm, host response, and implant factors. There is limited long term data on implant follow up due to the new implant surface treatments, and difficulty in following up patients over 5 years, which makes monitoring and studying disease progression a challenge for researchers. Earlier studies in literature often employed varying diagnostic methods and diverse inclusion/exclusion criteria, posing challenges for systematic reviews. Microbiome complexity presents diagnostic challenges. Pinpointing upregulated cytokines facilitates the recognition of pivotal phases and possible junctures for intervention. Comparing cytokine profiles between peri-implantitis and health, or to periodontitis can help researchers identify potential unique aspects of peri-implantitis and its distinct immunological pathways.

Efforts were made to standardize the criteria of subject selection to eliminate any confounding factors that might influence the immune response of subjects, such as smoking and diabetes, recent antibody intake, pregnancy, systemic inflammatory diseases, and having both healthy and implants with peri-implantitis.

In the absence of baseline data, parameter selection is crucial for distinguishing between peri-implant mucositis with biologic remodeling and peri-implantitis. In cases where baseline readings or radiographic documentation were not available, The AAP in the Consensus report of workgroup 4 of the 2017 World Workshop specified the bone loss levels of more than 3mm in addition to presence of bleeding on probing (Berglundh et al., 2017). In a subsequent publication, the 2017 WWP criteria demonstrated a high specificity but low sensitivity using the aforementioned approach and found the combination of BOP and bone loss of more than 2mm to be more accurate (Romandini et al., 2021). This parameter selection aligns with the VIII European Workshop on Periodontology (VIII EWP) that proposed the findings of a vertical distance ≥2 mm of bone loss in radiographs along with bleeding/suppuration on probing for diagnosing peri-implantitis (Sanz & Chapple, 2012). The diagnosis of peri-implantitis in this study adheres to this current definition.

The sample size of this study is relatively small to describe the expression of cytokine. Multiple, independent centers, with a larger sample size is needed to confirm these results. The extensive inventory of the salivary proteome, juxtaposed with the plasma proteome, offers valuable insights that can guide forthcoming investigations, particularly in the search for potential biomarkers for disease diagnosis (Yan et al., 2009). Saliva was used in our study for the following reasons: simple procedure to encourage

recruitment of patients, noninvasive collection procedure that requires no special equipment or facilities, ethically causes no harm to patient and allows recruitment from a larger pool of patients, allows the collection of a large sample for use in other studies in our lab, and to facilitate repetition of our study by other researchers with ease. Storage is simpler to achieve, and contamination risk is lower without the need for specially trained personnel, making saliva an attractive biofluid for disease detection (Yan et al., 2009). Tissue harvest procedure is more invasive, requires anesthesia, surgically trained providers, advanced facilities for proper storage and handling, carries a risk of infection, bleeding, discomfort, and would most likely yield a one-time sample. Recruitment of patients is more difficult as a result and could result in loss of attachment. Collection of crevicular fluid is more difficult than saliva due to small sample size collection, more technique sensitive, contains less protein content, and would provide results that provide information specific to the local site of collection.

Within the limitation of this study, there was no significant difference between the inflammatory cytokine levels of IL-18 in non-smoking patients and those in healthy patients. Future studies on the contribution of cytokines to peri-implantitis pathogenicity are also needed. Strategies to target cytokines may be critical for per-implant health and the long-term survival of implants. Another future direction is to confirm the results of this study using other methods and to identify distinctive mediators potentially contributing to disease progression such as using crevicular fluid, tissue harvest, or using genomic transcriptomic methods.

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