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The Effect of Periodontal Therapy on TNF-α, IL-6 and Metabolic Control in Type 2 Diabetics

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Purpose.This pilot study investigated if scaling and root planing (S&RP) was an effective intervention in reducing levels of inflammatory markers TNF-α and IL-6 in a type 2 diabetic population

Methods. Twenty-five patients with type 2 diabetes, 18-64 years of age were enrolled having 4 or more sites with pocket depths ≥ 5mm and 2 or more sites with attachment loss ≥ 3mm. Participants received S&RP following collection of gingival crevicular fluid and serum which were analyzed for TNF-α and IL-6. After 3 months post-treatment levels were collected. Serum pre-and post-treatment levels were analyzed using a paired t test at a significance level of p≤ 0.05. Mean TNF-α was 1.7pg/ml at baseline and post-treatment was 4.0pg/ml. Mean IL-6 was 2.8pg/ml at baseline and post-treatment 6.0pg/ml.

Results. Both mean TNF-α and IL-6 were increased following S&RP ; however, the observed increases were not statistically significant. While participants improved on periodontal measures following therapy, systemic measures of inflammation (TNF-α and IL-6) did not show the hypothesized reductions.

Conclusion. Further studies are needed to determine effectiveness of S&RP on inflammatory mediators in a population with type 2 diabetes.

Keywords: type 2 diabetes, periodontitis, periodontal therapy, scaling and root planing, inflammatory mediators

Introduction

Diabetes is a metabolic disorder resulting in chronic hyperglycemia and hyperlipidemia that ultimately induces diverse multiple system pathologies, increasing the risk for atherosclerosis, coronary heart disease, stroke, myocardial infarction, renal disease and periodontitis.[1-5] Diabetes has become the fifth leading cause of death affecting approximately 17 million individuals or 6.2% of the population in the United States.[5,6] If left untreated or uncontrolled, diabetes will lead to heart disease, stroke, blindness, amputations, kidney failure, periodontal disease and death.[7]

Periodontitis is an infection caused by the gram-negative organisms in the plaque biofilm that affects 7% to 15% of the adult population.[8,9] An abnormal inflammatory response that is a hyper-inflammatory trait has been linked to diabetes where there is an increased susceptibility to infections such as periodontal disease.[10,11] The hyper-inflammatory trait is associated with an exaggerated secretion of inflammatory mediators (TNF-α and IL-6) and systemic markers of inflammation. It is suggested that this process mechanistically contributes to the pathology associated with this chronic disease process.[2-4,8,12,13]

Diabetes and chronic periodontitis are both common chronic diseases observed in a significant proportion of the adult US population.[3,4] It is well established that diabetes is a risk factor for poor periodontal health; however, recent studies have also suggested that periodontal disease adversely affects glycemic control in diabetics.[1-5,8,12] Diabetic complications have been attributed to the hyperglycemic state, which over time results in the irreversible covalent modification (glycosylation) of structural proteins and lipids that comprise the extracellular matrix and connective tissues, as well as the vascular tissues.[14-16] These structural changes result in impaired capillary function, poor blood perfusion of tissues and organs, and the release of reactive oxygen species (oxidative stress) triggering a systemic inflammatory process.[12,17] The activation of inflammation at a systemic level results in the chronic elevation of inflammatory mediators (IL-1, TNF-α, IL-6, and PGE_2) and acute phase reactants such as C-reactive protein, elevated fibrinogen, and lowered albumin, all hallmarks of the acute phase reaction (APR) observed in diabetes and periodontitis.[17-19] Thus, a hyper-inflammatory trait may predispose an individual to a more severe systemic disease that may occur as a result of over expression of inflammatory mediators and may ultimately lead to metabolic dysregulation.

The purpose of this study is to determine if periodontitis serves as a stimulus for a systemic-based inflammatory response that may represent a previously underestimated metabolic stressor, enhancing insulin resistance and impairing insulin secretion. Further, looking at the effect of non-surgical periodontal therapy (scaling and root planing (S&RP)) in patients with type 2 diabetes on the inflammatory mediators TNF-α and IL-6 and the relationship of these mediators to markers of insulin resistance may provide evidence of the importance to a successful periodontal treatment outcome on oral-as well as systemic-health.

Review of the Literature

Diabetes

Diabetes is a metabolic disease due to disturbances in insulin production resulting in abnormal fat, sugar, and protein metabolism producing a hyperglycemic state.[20] Insulin, a hormone produced by the pancreas, normally is released in small amounts on a constant basis. When a meal is consumed, insulin is then released in greater amounts. The body has the ability to remove the excess glucose and stores it in the liver and muscle or converts it to fat. , When needed, this stored glucose is released back into the blood stream, where insulin ushers the glucose back into the cells. In a patient with diabetes, this process changes; excess glucose builds up in the bloodstream because of insufficient insulin released by the pancreas or cells resistant to insulin.[7] Type 2 diabetes usually develops over time and typically involves reduced responsiveness of tissues to circulating insulin. Diabetes is often controlled by diet or hypoglycemic agents.[2-4,8,12] If not

controlled through blood sugar monitoring, healthy eating, and weight control, type 2 diabetes can contribute to an increased susceptibility to infection and inflammation such as that seen in periodontal disease.[2-4,8,12]

Prolonged exposure to hyperglycemia is the primary factor for the development of diabetic complications. The biochemical basis is Advanced Glycation Endproducts (AGEs). AGEs are chemically irreversible, glucose derived compounds that form slowly and continuously as a function of blood glucose concentration. AGEs accumulate in plasma and tissues of diabetic patients.[21,22] Macrophages have an affinity for AGE and usually help normal tissue turnover by binding to receptor advanced glycation endproducts (RAGE (a macrophage receptor)) activating the synthesis of TNF-α and IL-1. If the synthesis and secretion is increased, as in hyperglycemia, then connective tissue degradation occurs.[23]

The Centers for Disease Control (CDC) has stated that the number of Americans with diabetes is on the rise.[5] It is the fifth leading cause of death in America with approximately 800,000 new cases annually.[5] Global estimates by Zimmet and McCarty predict diagnosis of non-insulin dependent diabetes (NIDDM) at 216 million by 2010.[24] In 1986, Huse et al estimated the economic burden of NIDDM to be 19.8 billion dollars in the United States.[25] Other studies have estimated costs for all diabetics in the U.S. (NIDDM and IDDM) at 100 billion.[6,20,25-28] Overall, there are enormous costs related to the treatment and control of diabetes in this country.

It is estimated that there are 5.5 million type 2 diabetic cases that may remain undiagnosed until symptoms prevail.[28] Women, American Indians, Asians, Hispanics, and African Americans have shown an increased prevalence of type 2 diabetes.[7] Most cases of newly diagnosed diabetes may have had the disease for four to seven years, suggesting that undiagnosed diabetes may have adverse effects even though in a quiescent state.[29] There are risk factors for diabetes, including, gender, race, family history, and a sedentary lifestyle.

Type 2 diabetes was previously described as non-insulin dependent diabetes and was once considered a late-onset disease. Currently it is increasingly found in a much younger population.[5]

Biofilm

Plaque formation is the primary etiology for inflammation in periodontal disease. Plaque is comprised of several hundred bacterial species.[30] Dental plaque is a microbial biofilm that is formed by organisms tightly bound to one another and to the solid substratum by means of an exopolymer matrix into which they are embedded.[31] The bacterium in biofilm consists of gram-positive coccoid cells that divide and form microcolonies. Periodontal bacterial pathogens such as *B.forsythus*, *P.gingivalis*, *T. denticola*, *C.rectus*, *P.intermedia* cause the tissue to breakdown and hinder the healing response, thus increasing probing depths, bleeding, and bone loss.[31] After a few days of dental plaque growth, filamentous bacteria coaggregate to the initial colonizers and become embedded in a matrix composed of salivary components and high proportions of exopolysaccharides of bacterial origin.[32] To maintain the ecosystem, anaerobes anchor to each other by forming an aggregated bacterial mass.[33] Biofilms are complex and yield a challenge in understanding the many interactions between bacteria and substrate, and the bacterial components found in mature plaque.[34] Biofilms occurring in nature are firm clusters of bacteria adhering in layers to some kind of substrate.[35]

Bacteria are anchored to the tooth surface via a three-dimensional plaque matrix where more bacteria cluster and infiltrate, damaging tissue and destroying bone. The pellicle, a condensate of salivary proteins, forms first and then the bacteria adhere to that layer.[36] The bacteria then proliferate and communicate with each other.[37] Biofilm can form on restorations, implants, and hard and soft tissues soon after tooth debridement. Biofilm is difficult to remove with regular saliva flow, tongue movement, and antimicrobial agents.[35]

Supragingival plaque is distinct from subgingival plaque because it starts supragingivally and then progresses subgingivally.[34] Subgingival plaque repopulates rapidly and has been hypothesized to cause periodontal disease.[38] With the plaque growing

subgingivally and the bacteria disrupting the health of the tissues, deciding the course of effective treatment is of prime importance.

Understanding the makeup of the bacteria in plaque may provide a broader look at how periodontal disease can be stopped or controlled. There are at least 4 different approaches that can be taken: preventing biofilm formation, disrupting existing biofilms, preventing further biofilm growth, and killing specific organisms in the biofilm.[31] With this knowledge, planning a course of treatment for patients with type 2 diabetes may enhance their oral health and help to control the effect of diabetes on periodontal disease.

Periodontal Disease

Periodontal disease is a chronic inflammatory condition of gingival tissues causing destruction of periodontal tissues and loss of alveolar bone by *P. gingivalis*, and other anaerobic gram-negative pathogens.[10,19] These pathogens produce endotoxin lipopolysaccharide (LPS), a component of the outer membrane of bacteria. Periodontopathic organisms exhibit a number of virulence factors that enable them to evade neutrophil clearance and establish themselves as chronic subgingival inhabitants. Among these is LPS.[39] It is believed that when increased amounts of LPS are released it causes macrophages and fibroblasts to over-produce the inflammatory cytokines IL-6, IL-1, and TNF-α. This leads to the progression of periodontitis, which includes destruction of periodontal tissues, inflammation, and bone resorption, causing an immune response.[8,9,40] Once the bacteria has invaded the host clearance system, the host becomes exposed to an array of bacterial toxins. The interaction of the bacterial toxins with mononuclear phagocytic cells results in activation of an inflammatory cascade, with synthesis and secretion of TNF-α, IL-6, IL-1, and PGE_2.[41] Even though NIDDM and IDDM have different origins of disease (environmental versus genetic), chronic hyperglycemia in the presence of LPS is adequate for monocytic hypersecretion of cytokines and periodontal disease progression. The breakdown of connective tissue and alveolar bone in periodontal disease result mainly from an infection mediated pathway of cytokine upregulation.[42]

Hyperglycemia and hyperlipidemia have been pathologically implicated in complications of diabetes and periodontal disease. It has been shown that advanced glycation endproducts, which have formed as a result of hyperglycemia/hyperlipidemia, can alter the phenotype of cell types by receptor advanced glycation endproducts, a cell surface receptor. AGE then binds to RAGE and transforms macrophages into cells with a destructive phenotype producing inflammatory cytokines IL-1, IL-6, and TNF-α.[2-4, 8, 12] Periodontal infection-mediated cytokine synthesis and secretion may amplify the magnitude of the AGE-mediated cytokine response. As a result of the AGE/RAGE complex, there is a two-way relationship between diabetes mellitus and the infection caused by periodontal disease.[43]

Cytokines (IL-1, IL-6, and TNF-α) are soluble, biologically-active glycoproteins secreted by host immuno-inflammatory cells.[40] They have a role in the inflammatory process and are produced by lymphocytes, monocytes, macrophages, granulocytes, epithelial cells, endothelial cells, adipose tissue and fibroblasts.[44] Elevation of inflammatory mediators IL-6, IL-1, and TNF-α causes dysregulation of lipid metabolism and insulin resistance, thus breaking down gingival tissue, enhancing bone resorption by signaling osteoclasts and adding to long-term complications in the patient with diabetes.[8]

Acute infection results in the systemic challenge of pyrogenic cytokines, such as IL-1, TNF-α, and IL-6, which block lipoprotein lipase activity, resulting in decreased transportation of blood lipids from the circulating cells, eliciting hyperlipemia.[17,45,46] TNF-α promotes glycogenolysis and impairs glucose uptake by cells in the periphery, presumably by an effect on glucose transport receptor expression leading to hyperglycemia.[17,46] TNF-α and IL-6 target the hepatocyte to induce acute phase response (APR).[17,45,46] It is believed that these cytokines are detected in a periodontal lesion and can be heightened by the onset of diabetes.

Diabetes and Periodontal Disease

Historically, patients with diabetes have been shown to be at increased risk for infections. Increased periodontal risk is often related to the duration and adequacy of control of the diabetic state. It has been noted that individuals with type 2 diabetes have a three-fold increased risk of developing periodontal disease that can not otherwise be explained on the basis of age, sex, or oral hygiene.[47] Past and present studies have reported periodontal disease to be one of the most prevalent complications of diabetes.[2-4,48] The classic presentation of periodontal disease progression has been associated with accumulation of plaque and calculus on the tooth surfaces, and potent virulence factors produced by bacteria, causing destruction of periodontal tissues and resorption of alveolar bone.[10] Patients with diabetes have a compromised host response and ability to respond to bacterial infections, which in part, may increase their risk of periodontal disease. The reverse of this theory is that periodontal infections may exacerbate the diabetic condition.[42] Studies demonstrating the relationship between diabetes and the association of microbial organisms in prevalence and severity of periodontal disease show that the flora associated with diabetes does not appear to differ from patients without diabetes.[49] Patients who poorly control their diabetes and have periodontitis show an increase in progression of periodontitis. Patients who control their diabetes, receive timely care and control their blood sugar, are no more likely to develop severe periodontal disease than patients without diabetes.[2-4] The literature clearly supports that diabetes increases the risk for severe periodontitis and an increased incidence of periodontal disease progression by approximately 2 to 3 times than that observed in healthy patients.[2-4,12,50,51] Traditionally, these complications have been attributed to the hyperglycemic state, which over time, results in glycosylation of structural proteins and lipids that comprise the extracellular matrix and connective tissues, as well as the vascular tissues. These structural changes result in impaired capillary function and poor blood profusion, triggering a systemic inflammatory process. The activation of inflammation at a systemic level results in the chronic elevation of C-reactive protein (CRP), a hallmark of APR.[17,45,46] Studies of patients with diabetes typically demonstrate an elevation of APRs, which tend to correlate with the degree of glycemic control.[17,18,45,46] Thus, it has been generally hypothesized that elevated APR markers in type 2 diabetes are a direct consequence of diabetic metabolic dysregulation.[52] This suggests that periodontal disease may actually contribute to the development of metabolic imbalance, which may result in insulin resistance or impair insulin secretion and type 2 diabetes.[52] In a longitudinal study, participants with type 2 diabetes and severe periodontal disease at baseline demonstrated significantly worse glycemic control than participants with diabetes who have minimal periodontal destruction.[49]

Studies have shown that the Pima Indians, a population who have a high incidence of type 2 diabetes, rank as one of the highest populations in the world with this systemic disease.[53] They also have a high prevalence of periodontal disease.

Current Therapies for Treatment

Preventing dental plaque (biofilms), which holds the bacteria and causes destruction of the periodontal tissues, can be achieved by inhibiting the attachment of bacteria, altering the structure of deposit, or interfering with the pattern of plaque development.[54,55] Once the plaque deposits have formed, primary prevention could be accomplished through reducing existing plaque, preventing the formation of new plaque, selectively inhibiting particular bacteria associated with disease, or inhibiting the expression of virulence determinants.[56] Periodontal disease is a plaque-induced infection. Patients are generally not skilled at removing the plaque and are unable to remove subgingival deposits of plaque and calculus; thus, professional debridement and scaling is needed to maintain the health of the periodontium.[57] Not everyone responds to therapies or is able to maintain the health of the periodontium after S&RP. There are many reasons why traditional therapies do not control disease such as poor compliance with home care, patients not returning for regular maintenance visits, insufficient debridement by the clinician or reinfection by the bacteria, and above all, systemic diseases such as type 2 diabetes.[42,58-60] By maintaining regular visits for oral care and proper home care, the microbiota can be kept under control and damage to the periodontium will be decreased. Studies show that a reduction in inflammation after periodontal treatment will reduce the insulin a patient requires.[61] By including a therapeutic approach to reducing the bacteria the health of the diabetic patient may improve and reduce blood sugar levels.

Anti-infective therapy includes both mechanical and chemotherapeutics to eliminate or decrease biofilms. Mechanical therapy, supra- and subgingival scaling and root planing, consists of debridment of the roots by hand or power-driven scalers to remove plaque, endotoxin, calculus, and other plaque-retentive local factors.[62] Mechanical S&RP to remove plaque and calculus is essential to decreasing the inflammatory response. This therapy is increasingly important for patients with type 2 diabetes who have an increased susceptibility to inflammation.[38] Non-surgical periodontal therapies, such as S&RP and S&RP with antimicrobial therapies, have been shown to decrease periodontal disease progression.[42, 58] By using manual or sonic driven scalers in subgingival pockets there is a profound shift in the composition of microbial flora.[63-65] By effectively removing the endotoxins in the subgingival areas, healing of the tissues can occur and a reduction in probing and attachment levels can be attained.

To date, no one treatment has been truly successful. In a study by Christgau et al, HbA1c levels were measured prior to and after S&RP. The treatment group received S&RP and gingival curettage while the control group did not receive any treatment. In the treatment group the levels of HbA1c were measured pre- and post- treatment and then again at 9 months; HbA1c levels improved by 6.7% in the control group and 17.1% in the treatment group. This study reported that mechanical therapy had no effect overall on levels of HbA1c.[66] A study by Westfelt et al looked at the maintenance of patients with diabetes 5 years after S&RP and found no alterations in HbA1c levels.[67] Rodrigues et al found that S&RP and S&RP in combination with amoxicillin/clavulanic acid reduced HbA1c levels, especially in patients who had an increased severity of diabetes and periodontal disease. The HbA1c levels were taken at baseline and after 3 months following therapy. Both groups improved but the group with antimicrobial therapy had a greater reduction in HbA1c values. Thus, the use of antimicrobial therapy along with S&RP was found to improve the levels of glycemic control in patients with type 2 diabetes.[44] Stewart et al did a retrospective study and found that there was an improvement in glycemic control in participants with type 2 diabetes mellitus following S&RP.[68] Antimicrobial therapy, minocycline gel, when introduced along with S&RP, decreased TNF-α, subgingival bacteria, and HbA1c levels; probing and attachment levels were also reduced.[69] There are no studies where the levels of TNF-α and IL-6 have been assessed following S&RP. Most studies that investigated S&RP and its effect on patients with type 2 diabetes' oral health did show that S&RP alone improved probing and attachment levels but they did not assess inflammatory mediator levels.[43,66,67] Many of the studies that investigated TNF-α and IL-6 levels studied adjunctive therapies with antimicrobials.[44,66,70] Thus, there is a need to look at the effect S&RP has on decreasing the levels of inflammatory mediators.

Investigators are also taking a closer look at the role of glycemic control. While it is well established that S&RP can influence levels of HbA1c, what is not clear is how S&RP influences levels of inflammatory mediators. For example, TNF-α has been identified as a strong antagonist to the cell surface insulin substrate.[71] This activity, by TNF-α blocking of the insulin receptors, can contribute to the level of insulin resistance by inhibiting glucose transport and insulin action.[19] Recent evidence has suggested that chronic infection via periodontitis can influence insulin resistance.[50,72] Therefore, it is hypothesized that toxins from subgingival bacteria can produce chronic increases in inflammatory mediators such as TNF-α, which have been implicated in patients with type 2 diabetes and inflammation. This increase in inflammatory mediators is believed to increase the severity of diabetes and negatively influence diabetic control.

The overall objective of this pilot study was to improve the understanding of the mechanisms by which infection contributes to the metabolic dysregulation associated with the diabetic state and to provide primary prevention strategies. The magnitude for additional research initiatives and clinical interventions for diabetes is very apparent with expenditures climbing higher and higher and no cure available. Years of research has produced evidence about the increased frequency and severity of periodontal disease in patients with type 2 diabetes.[2-4,8,12,48] Recent studies have reported that a controlled periodontal condition may positively influence a patient with diabetes' glucose level.[2-4,8,12] If this is the case, it is of great interest to explore the inflammatory response in the patient with diabetes, mechanisms to control it, and the possibility that reduction of periodontal disease through mechanical therapy might lead to a better control of blood glucose. This research will contribute to the body of knowledge within the national dental hygiene research agenda for promoting health in a population with type 2 diabetes. It will also enhance the knowledge of how inflammation affects diabetes, specifically insulin resistance

and inflammatory mediators TNF-α and IL-6, and the long term effects that this plays on total body health. In obtaining a greater understanding of diabetes and periodontal disease, dentists and hygienists can better inform patients with type 2 diabetes about nonsurgical periodontal therapies.

Methods and Materials

Twenty-five participants with type 2 diabetes were recruited through advertisements and e-mail messages. All participants had to have type II-IV periodontal disease as defined by the American Academy of Periodontology (AAP).[73] Participants could not have had periodontal therapy (scaling and root planing or periodontal surgery) within 6 months prior to enrollment in the study.

Participants were first appointed for a screening visit to determine eligibility in the study. If eligible, a full-mouth series of x-rays (FMX) were taken to determine bone loss. Participants were then appointed for three additional visits. Inclusion and exclusion criteria were as follows.

Inclusion Criteria:

1. 4 or more sites with probing pocket depths ≥ 5mm.
2. 2 or more sites with attachment loss ≥ 3mm.
3. 18-64 years of age.

Exclusion Criteria:

1. < 20 teeth.
2. Systemic disease (systemic lupus erythematosis, HIV, AIDS).
3. Immunosuppressive Therapy (Cortisone, steroids, cancer chemotherapy).
4. Recent periodontal surgery or scaling and root planing (S&RP) within the past 6 months.
5. Chronic liver disease including Hepatitis.
6. BMI ≥ 40.
7. Pregnant.
8. Current abuse of alcohol or drugs.

Procedures

Medical and dental information was collected at the screening visit and updated at each subsequent appointment. At visit 1 and visit 2, participants fasted for a minimum of 6 hours. Blood was collected for serum to analyze TNF-α and IL-6, HbA1c, fasting insulin, and glucose. Gingival crevicular fluid (GCF) was collected to assess TNF-a and IL-6. The periodontal evaluation consisted of plaque index (PI),74 gingival index (GI),74 probing depths (PD), bleeding on probing (BOP), and clinical attachment level (CAL) measured from cementoenamel junction (CEJ) to gingival margin (Figure 1). After visit 1, participants were reappointed for the treatment visit (S&RP) in which anesthetic was administered by the dentist, if the patient requested it. After treatment was completed, participants were reappointed for visit 2; this occurred 3 months after treatment (S&RP) was completed. Visit 2 consisted of the same treatment as in visit 1. Oral hygiene instructions were not provided and compliance with home care was not followed. The following is a list of all the procedures that were conducted during each appointment (Figure 2).

Figure 1.

Clinical Indices Performed on each Participant

1. Plaque Index (PI): supragingival plaque recorded on an ordinal scale of 0-3[74]
2. Gingival Index (GI): the degree of buccal inflammation recorded on an ordinal scale 0-1[74]
3. Probing Depth (PD): the distance from the free gingival margin to the base of the pocket measured in millimeters
4. Bleeding on Probing (BOP): the presence or absence of bleeding recorded as 0 (no bleeding) or 1 (bleeding present)
5. Clinical Attachment Loss (CAL): measurement from the cementoenamel junction to the gingival margin
6. Gingival Crevicular Fluid (GCF): a fluid that is found in small amounts in the gingival crevice.

Figure 2

Screening

1. Determined if patient was eligible for the study through probing depths and CAL
2. Informed consent, HIPPA
3. FMX

Visit 1*

1. Fasting blood draw for serum TNF-a and IL-6, HbA1c, fasting insulin and glucose
2. Probing Depths (PD)
3. Clinical Attachment Loss (CAL)
4. Gingival Index (GI)74
5. Bleeding Index
6. Plaque Index74
7. Oral Examination (caries, oral lesions, missing teeth)

*Collected by examiners

Treatment

1. 1. Treatment (S&RP) with or without anesthetic

* Registered dental hygienist completed S&RP

Visit 2*

1. Same as visit 1.

*Collected by examiners

This study utilized three examiners who were all calibrated to the gold standard and to each other as set forth by University of North Carolina-Chapel Hill School of Dentistry and based on the kappa statistic.[75] Two examiners were research dentists (examiner 1 and examiner 2). A dental hygienist with 7 years of clinical experience performed all scaling and root planing procedures. The dental hygienist (clinician) and examiner 2 performed all treatment (S&RP). The examiners were calibrated to themselves (intra-rater) as well as to a gold standard and to each other (inter-rater), and percent agreement and kappa scores were determined.

Gingival Crevicular Fluid

GCF was taken at visit 1 and visit 2 in 4 quadrants starting with the distal of the second premolar, then the mesial and distal of the first molar and then the mesial of the second molar. If these teeth were unavailable, the teeth in succession with each other were utilized (the next more mesial tooth in proximity). Each site was assayed for TNF-α and IL-6. Each site was sampled using sterile cotton forceps and a strip of PerioPaper (Ora Flow, Inc. Plainview, New York). The PerioPaper was grasped by the orange strip and inserted into the gingival sulcus until a resistance was felt. Care was taken to ensure that contamination by food or blood was limited. The PerioPaper remained in the sulcus for approximately 10 seconds or until the paper was beginning to look saturated. The PerioPaper was removed from the sulcus and inserted between the calibrated Periotron (Ora Flow, Inc., Plainview, New York) sensors. The sensors were closed and a reading was obtained after a 16-second measuring cycle. The reading obtained was between 30 and 180. Any readings that were below 30 or over 180 were thrown out and a new reading was obtained. After the proper reading was obtained, the PerioPaper was wrapped in aluminum foil to prevent evaporation. The wrapped aluminum foil was placed in a cryovial, which had been labeled for each patient enrolled in the study and then immediately placed in a canister containing liquid nitrogen. The canister of liquid nitrogen was used to transport the GCF samples to the Center for Oral and Systemic Disease, where the samples were stored at -80°C until they were analyzed.

Results

Patient Population

Upon approval by the University of North Carolina School of Dentistry Institutional Review Board, 28 participants were enrolled in the study. However, 2 participants did not return for post-evaluation, one subject did not complete the initial clinical evaluation and collection (visit 2), and one patient did not fast for visit 2; thus, complete data was collected on 23 participants. Nevertheless, because some data was collected on 25 participants, some variables will have an N =25. See Table 1.

Table I. Demographics of Participants

		n	Percent	Mean/SD
Gender	Male	12	48%	
	Female	13	52%	
Race	African Am.	14	60.87%	
	White	9	39.13%	
BMI		24		33.05±7.03
Age (years)		25		49.92±8.41

Clinical Measurements

A paired *t* test was used to determine significance in the mean change between the baseline and post-treatment values for all clinical parameters. Significant improvements were found in all clinical parameters. Mean probing depths at baseline were 3.1 and post-treatment was 2.4 (p=0.02). Mean attachment loss at baseline was 3.0 and post-treatment 2.4 (p=0.05). Mean plaque index was 0.77 at baseline and 0.44 at post-treatment (p=0.01). Mean gingival index was 1.1 at baseline and 0.85 at post-treatment (p=0.04). Mean bleeding on probing was 0.57 at baseline and 0.42 at post-treatment (p=0.0005). (See Table II and Figure 3.)

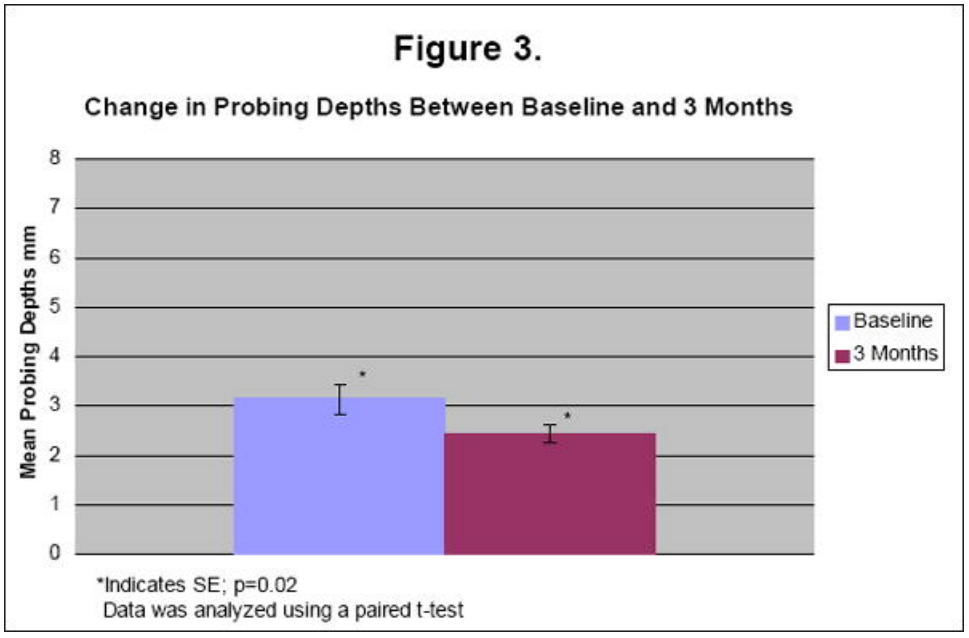


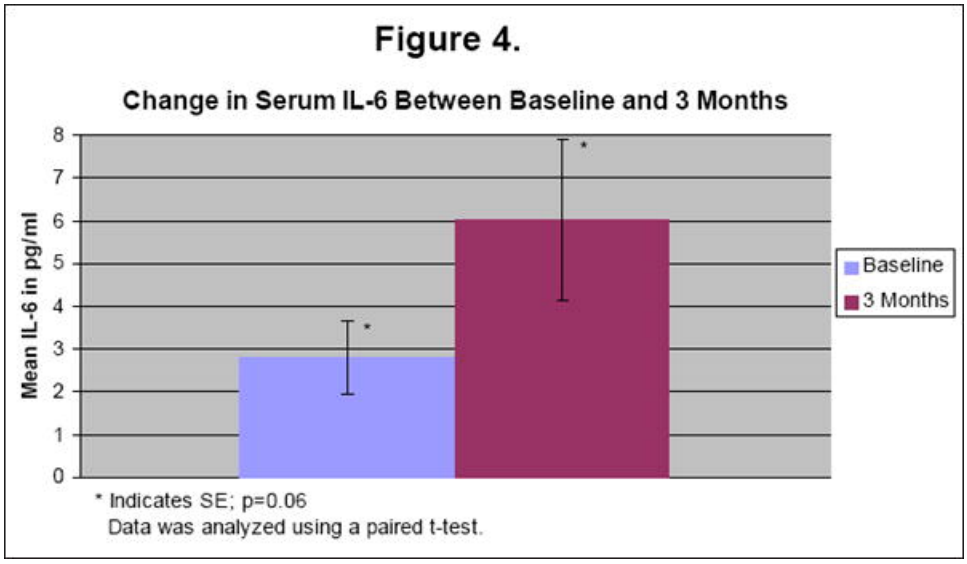
Table II. Baseline and Post Treatment Values For Outcome Variables

Baseline and Post-Treatment Values For Outcome Variables				
	n	Baseline Mean/SD	3 Months Mean/SD	P value
TNF-α	21	1.7±4.34	4.0±13.6	p=0.32
IL-6	23	2.7±3.9	6.0±9.0	p=0.06
HbA1c	25	8.2±1.8	8.3±1.9	p=0.66
Fasting Insulin	25	18.5±18.6	21.2±16.1	p=0.33
Fasting Glucose	25	177±66.9	193±70.0	p=0.24
Homa	25	134±126	161±128	p=0.34
Attachment Loss	24	3.0±1.6	2.4±1.1	p=0.05*
Plaque Index	24	0.77±0.55	0.44±0.35	p=0.01*
Gingival Index	24	1.10±0.58	0.85±0.63	p=0.04*
Bleeding on Probing	24	0.57±0.30	0.42±0.25	p=0.0005*
Probing Depth	24	3.1±1.5	2.40±0.90	p=0.02*

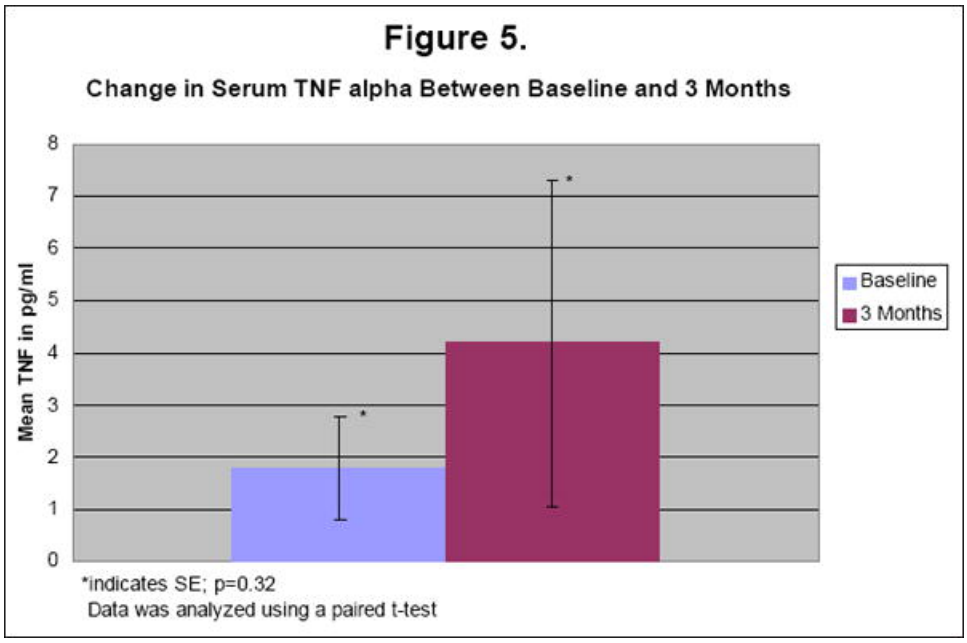
*alpha=0.05; Data was analyzed using a paired t-test

Serum TNF-α and IL-6

Mean IL-6 levels at baseline were 2.8 pg/ml and post-treatment levels were 6.0 pg/ml. Statistical analysis using a paired t test was used to look at the change in serum IL-6 levels from baseline to post-treatment. The observed increase in IL-6 was not significant (p=0.06). (See Figure 4.)



TNF-α mean levels at baseline were 1.8 pg/ml and post-treatment 4.2 pg/ml. Statistical analysis was done using a paired t test to look at the change in serum TNF-α levels from baseline to post-treatment. No significance was shown p=0.32. (See Figure 5.)



Discussion

This study attempted to assess the effect non-surgical periodontal therapy (scaling and root planing (S&RP)) would have on inflammatory mediators TNF-α and IL-6 and the relationship of these mediators to markers of insulin resistance. The results of this pilot study showed non-significant increases in TNF-α or IL-6 pre- and post-treatment. Statistical significance was shown in reduction of pocket depth, gingival index, attachment loss, plaque index, and bleeding on probing.

Patients with uncontrolled type 2 diabetes with periodontal disease are systemically stressed by the bacteria in plaque, the increase in circulating inflammatory mediators, and an increase in insulin resistance. In this study the clinical periodontal parameters improved, while the patients did not show improvement in measures of systemic inflammation (IL-6 and TNF-α). One plausible explanation for this apparent contradiction is that S&RP produced systemic stress because bacteria were released into the blood stream. As a result, the patient's systemic inflammatory system may have tried to combat the inflammatory process. The alternative explanation for the improvement in periodontal clinical parameters while the systemic mediators appeared to be increasing is that the observed results occurred purely by chance.

In this study the mean Body Mass Index (BMI) for participants was 33. Because TNF-α and IL-6 are produced in the adipose tissue, the higher BMI may have increased the participants metabolic stress, which could have caused the elevation in TNF-α and IL-6. Visceral obesity is quite common in patients with type 2 diabetes and could be a contributing factor to why there was not a reduction in these mediators nor HbA1c levels. Nishimura et al studied levels of TNF-α in correlation to obesity levels of participants and found that there was a correlation between weight and mediator levels.[50]

The addition of an adjunctive antimicrobial agent to the S&RP regimen may have produced a different effect on the bacteremia theory. Previous studies of patients with type 2 diabetes have shown positive results with the addition of local and systemic drugs which decreased the serum levels of TNF-α and IL-6. Iwamoto's study reported a decrease in serum levels of TNF-α, circulating insulin concentrations and HbA1c levels in a diabetic population by placing 10 mg of local minocycline in every pocket and mechanical debridement of plaque once a week for a month.[69] As Grossi and Genco reported in a review of the literature, S&RP alone showed improvement in periodontal status but when systemic antibiotics plus S&RP were incorporated there was an improvement in blood glucose levels, a decrease in gram-negative bacterial levels and a reduction in inflammatory mediators.[42]

In future studies, having a larger patient sample size to assess serum levels of IL-6 and TNF-α could possibly increase the significance of serum TNF-α and IL-6. In this study, serum was assessed prior to scaling and root planing and then again 3 months after scaling and root planing. By having a shorter sample time period between treatment and re-sampling, more information could be provided on TNF-α and IL-6 as it relates to patients with type 2 diabetes.

In addition to having a shorter interval between S&RP and post-treatment, oral hygiene instructions may have enhanced or provided more significant results. Most patients have poor compliance or lack the motivation needed to succeed with oral hygiene aids.[58] By providing reinforcement of oral hygiene aids prior to, during, and post- S&RP, the patient may begin to develop the understanding of how important regular home care is and its role in decreasing the progression of periodontal disease thus controlling the diabetic state.

A control group was not utilized in this pilot study. Control groups decrease error and reduce bias. A control group is an asset to any study because it provides data on a healthy population that can be compared with a population with disease. The present study sought to collect pilot data on the effects of S&RP on inflammatory mediators TNF-α and IL-6 in a population with type 2 diabetes. A future study will incorporate an experimental and control group to assess the differences.

This study has shown that further investigations are needed to look at what role TNF-α and IL-6 have on the markers of insulin resistance in the population with type 2 diabetes. In future studies, increasing the sample size, adding a control group, having a run-in period where patients would serve as their own control to show stability of the measures during the run-in period, and the addition of additional therapies would be an enhancement to the study design.

Conclusion

It has been recognized that scaling and root planning (S&RP) is an effective therapy in reducing pocket depths and decreasing bleeding on probing, thus restoring periodontal health to the patient and then in turn decreasing insulin resistance.

This pilot study investigated the effect of S&RP in patients with type 2 diabetes on inflammatory mediators TNF-α and IL-6 and the relationship of these mediators to markers of insulin resistance. A second objective was to determine if periodontitis serves as a stimulus for systemic-based inflammatory response that may represent a previously underestimated metabolic stressor, enhancing insulin resistance and impairing insulin secretion. At visit 1 and visit 2, clinical assessments along with gingival crevicular fluid, and serum samples were taken to examine TNF-α and IL-6 levels.

The results of this study show that S&RP is a beneficial treatment in decreasing the clinical periodontal parameters; but, in this population, systemic reduction of TNF-α and IL-6 was not significant. These results could have been affected by an already systemically stressed population of participants. Performing S&RP could have increased the participants' systemic stress load through the release of bacteria into their bloodstream.

Further studies are needed to assess the effect of non-surgical periodontal therapy to inflammatory mediators TNF-α and IL-6 and how they affect the markers of insulin resistance in patients with type 2 diabetes.

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Notes

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