DİABETUS MELLİTUS TİP 2 TEŞHİSİ KONULMUŞ BİREYLERDE TNF-α, IL-β, IL-6, MMP-8 VE MMP-9 DÜZEYLERİNİN SİSTEMİK SAĞLIKLI BİREYLERLE KARŞILAŞTIRILMASI

COMPARISON OF TNF-α, IL-β, IL-6, MMP-8, AND MMP-9 LEVELS IN PATIENTS WITH AND WITHOUT DIABETES MELLITUS TYPE-2

[1]*Abdullah Seçkin ERTUĞRUL, [1]Hacer ŞAHİN, [1]Ahu DİKİLİTAŞ, [1]Nazlı Zeynep ALPASLAN, [1]Alihan BOZOĞLAN, [2]Murat ESKİTAŞÇIOĞLU

[1]Yüzüncü Yıl University, Faculty of Dentistry, Department of Periodontology, Van, TURKEY
[2]Yüzüncü Yıl University, Faculty of Dentistry, Department of Prosthodontics, Van, TURKEY

Özet

Sitokinler, çeşitli hücreler tarafından üretilen enflamasyonun güçlü mediatörleri olarak tanımlanmaktadır. İnterleukin-1beta (IL-1β), IL-6 ve Tümör nekrosis factor-alfa (TNF-α) periodontal enflamatuar süreçte önemli rolü olan sitokinlerdir. Matriks metalloproteinazlar (MMPs) hücreler arası matriks yenilenme ve yıkım arasındaki dengeyi belirleyen enzimlerdir. Arteriyel hipertansiyon, akut koroner hastalık tablosu, kalp yetmezliği, böbrek hastalıkları, diabetus mellitus ve periodontal hastalıklar gibi patofizyolojik durumlarda plazma sitokinlerin ve enzimlerin seviyesinin yükseldiği saptanmıştır. Çalışmamızda dişeti oluğu sıvısında, IL-1β, IL-6, TNF-α, MMP-8 ve MMP-9 seviyesini tip 2 diabetus mellitus kronik periodontitisli bireyler ile sistemik sağlıklı kronik periodontitisli bireylerin karşılaştırılması amaçlanmıştır.

Çalışmaya toplam 50 birey dâhil edilmiştir. Çalışmaya katılan bireylerin 25 tanesi kronik periodontitis, 25 tanesi kronik periodontitis diabetus mellitus tip 2 tanılı bireylerden oluşmaktadır. Çalışmaya katılan bireylerin periodontal durumu periodontal klinik indeksler kullanılarak belirlenmiştir. Enzyme-linked-immuno-sorbent yöntemi dişeti oluğunda IL-1β, IL-6, TNF-α, MMP-8 ve MMP-9 seviyesini belirlemek için kullanılmıştır.

Çalışma grupları arasında cinsiyet ve yaş verileri değerlendirildiğinde gruplar arasında istatistiksel olarak fark bulunmamıştır ($p>0.05$). Diabetus mellitus tip 2 kronik periodontitis grubundaki bireylerin klinik periodontal indeksleri kontrol grubundaki bireylerden istatistiksel olarak yüksek bulunmuştur ($p<0.05$). Diabetus mellitus tip 2 kronik periodontitisli bireylerde dişeti oluğu sıvısında IL-1β, IL-6, TNF-α, MMP-8 ve MMP-9 seviyeleri sistemik sağlıklı kronik periodontitisli bireylerden istatistiksel olarak yüksek bulunmuştur ($p<0.05$).

Diabetus mellituslu bireylerde geri dönüşümsüz hasarlar gözlenmektedir. Diabetus mellituslu bireylerde periodontal dokulardaki yıkım daha şiddetli olmaktadır. Yıkımın artması ve mikroorganizma profilindeki değişimler nedeniyle diabetus mellituslu ve periodontal hastalıklı bireylerde dişeti oluğu sıvısında artmış IL-1β, IL-6, TNF-α, MMP-8 ve MMP-9 seviyeleri gözlenmiştir. Dişeti oluğu sıvısında IL-1β, IL-6, TNF-α, MMP-8 ve MMP-9 seviyelerinin belirlenmesi, periodontal hastalıkların ve diabetus mellitusun ilişkisinin tanımlanabilmesine yardımcı olacağı düşünülmektedir.

Anahtar kelimeler: TNF-α, IL-1β, IL-6, MMP-8, MMP-9, Diabetus mellitus

Abstract

Interleukine-1beta (IL-1β), IL-6, and tumor necrosis factor-alpha (TNF-α) are the cytokines that play a significant role in the inflammatory process. Matrix metalloproteinases (MMPs) are the enzymes that identify the balance between intercellular matrix renewal and destruction. The aim of this study is to compare the levels of IL-1β, IL-6, TNF-α, MMP-8, and MMP-9 in gingival crevicular fluid (GCF) in patients with and without type-2 diabetes mellitus (DM) chronic periodontitis.

A total of 50 patients were enrolled in the study. Twenty-five of the patients included in the study were systemically healthy-chronic periodontitis, and the other 25 were type-2 DM-chronic periodontitis. Enzyme-linked immuno sorbent method was used to compare IL-1β, IL-6, TNF-α, MMP-8, and MMP-9 levels in GCF.

Clinic periodontal parameters of patients in type-2 DM-chronic periodontitis group were statistically higher than systemically healthy-chronic periodontitis patients. IL-1β, IL-6, TNF-α, MMP-8, and MMP-9 levels in the GCF of patients with type-2 DM-chronic periodontitis were statistically higher than systemically healthy-chronic periodontitis patients.

The destruction in periodontal tissues is more severe in patients with DM. Due to the increase of destruction and changes in microorganism profile, increased levels of IL-1β, IL-6, TNF-α, MMP-8, and MMP-9 were observed in the GCF of patients with DM and periodontal disease. It is suggested that the identification of IL-1β, IL-6, TNF-α, MMP-8, and MMP-9 levels in GCF may enable the development of potential methods to treat periodontal diseases and DM.

Key words: TNF-α, IL-1β, IL-6, MMP-8, MMP-9, Diabetes mellitus

INTRODUCTION

The most common endocrine disease, DM, a metabolic disorder characterized by hyperglycemia, occurs due to the interaction of some environmental factors such as a high-fat diet, genetic history, and obesity. DM is classified into two categories: type-1 and type-2 DM. Type-1 DM (T1DM) occurs due to a reduction in insulin production as a result of autoimmune destruction of pancreatic cells, and is observed mostly in children and young adults. Type-2 DM (T2DM) is generally observed in adults and is characterized by the reduction in insulin resistance due to the failure in pancreatic beta cells to create a sufficient

***Corresponding Author**
Dr. Abdullah Seçkin ERTUĞRUL
Yüzüncü Yıl University Faculty of Dentistry
Department of Periodontology
65080 Van, TURKEY

e-mail: ertugrulseckin@yahoo.com, ertugrul@yyu.edu.tr

amount of insulin secretion. T2DM constitutes 70-95% of DM patients.[1, 2] IL-1β levels in GCF are lower in T2DM patients with gingivitis or slight periodontitis than in patients with moderate or severe periodontitis. Also, the levels of IL-1β in GCF in T2DM patients were higher than in systemically healthy patients.[3] Clinically healthy DM patients with pocket depth of not more than 3 mm have a more common form of periodontitis and have higher levels of prostaglandin E2 and TNF-α than systemically healthy patients.[4] Microorganism toxins are known to stimulate connection epithelium cells to secrete various inflammatory mediators in IL-1β, IL-6, TNF-α, and matrix metalloproteinases (MMPs). All of these mediators may pass over the connection of epithelium and reach the GCF. The normal flora of the body blocks the development of microorganisms and may act as an effective buffer against infections.[5] Cytokines that pioneer inflammation, such as IL-1β, IL-6, and TNF-α, play a very significant role in the initiation, regulation, and prolongation of natural immune response.[6] These cytokines cause vascular changes and the migration of cells such as from neutrophilia to periodontium. It is revealed that IL-1β, IL-6, and TNF-α have various activities that may cause tissue destruction, including chronic inflammation such as periodontitis.[7] IL-1β, IL-6, and TNF-α are the basic mediators of chronic inflammatory disease, and have the potential to destroy tissue and initiate bone loss.[7, 8] It is revealed that IL-1β, IL-6, and TNF-α stimulate fibroblasts in cultures to produce collagenase.[7, 9] Moreover, osteoblasts suppress alkaline phosphatase expression and matrix synthesis, and inhibit bone construction.[10] IL-1, which is the most powerful inducer of bone demineralization, exhibits a synergistic impact with TNF-α in stimulating bone resorption, as well as significant changes in collagen tissue matrix.[11, 12] According to bone resorption trials, IL-1β is 100-fold more potent than TNF-α.[13] TNF-α molecules induce multiplication and differentiation of osteoclast pioneer cells and stimulate bone resorption by indirectly activating matured osteoclasts.[14] TNF-α also induces IL-6 production, which stimulates osteoclast formation, direct osteoclastic bone resorption, and T-cell differentiation.[8]

In the formation of inflammation, which is the primary indicator of periodontal disease, the main mediators that identify the balance between destruction and improvement are proinflammatory cytokines and enzymes. As the severity of periodontal diseases increases so does the destruction of periodontal tissues. Inflammation is among the factors that trigger the destruction.[15] IL-1β, IL-6, TNF-α, MMP-8, and MMP-9 are supposed to play a significant role in the pathogenesis of DM and periodontal diseases. In the frame of these data, the aim of this study was to compare the levels of IL-1β, IL-6, TNF-α, MMP-8, and MMP-9 in the GCF of T2DM-chronic periodontitis patients with systemically healthy-chronic periodontitis patients by the enzyme-linked-immuno-sorbent (ELISA) method.

MATERIALS and METHODS

Individuals

The subjects of this study were patients who applied to be in the study at Yuzuncu Yil University, Faculty of Dentistry, Department of Periodontology between July 2011 and September 2011. A total of 50 patients were enrolled in the study. Twenty-five (12 males, 13 females) of these patients are diagnosed with systemically healthy-chronic periodontitis, while the other 25 (13 male, 12 female) are diagnosed with T2DM-chronic periodontitis (Table-1).

Patients enrolled in the research study met the following criteria: they did not use tobacco products, did not have any cardiovascular disease, did not have any chronic renal disease, have not had periodontal treatment within the last 6 months, have not taken any drug that contains antibiotics or that may impact immune system within the last 6 months (excluding DM medications), and have at least 20 teeth. The patients signed an approval form stating that they voluntarily participated in the survey. An approval was obtained from Yuzuncu Yıl University Faculty of Medicine, Human Research Ethics Committee (YYU-110411).

To evaluate the periodontal conditions of patients before the periodontal treatment, the records for plaque index (PI)[15], gingival index (GI)[16], probing pocket depth (PD), bleeding index in probing (BOP), and clinical attachment loss (CAL) were taken. During pocket depth measures, care was taken on parallel application of Williams periodontal probe to the

horizontal axis of teeth with its own weight without any pressure. In selecting patients with chronic periodontitis and periodontal health for the study, the focus was on the 1999 International Periodontal Workshop.[17]

Collection of Gingival Crevicular Fluid from Patients

GCF samples were taken from patients before periodontal treatment. Four samples were taken from four teeth that had the deepest pocket formations. Before sampling the surface where sampling would be done was isolated with cotton roll buffers and dried with pressurized air. Supragingival plaque was removed with a curette with no contact to gingival. Care was taken not to contaminate the paper strips (Periopaper Oraflow, NY, USA) with blood and saliva. Paper strips contaminated with blood and saliva were not included in the study. Care was taken not to create a mechanical trauma while placing the strips inside the gingival pocket until a slight resistance was felt. To provide standardization, strips were measured as µl with electronic equipment (Periotron 8000, Oraflow, NY, USA) for the GCF volume of each strip following a holding period of 30 seconds inside the GCF. Collected strips were placed in eppendorf tubes and stored under –80 ºC.

Identification of IL-1β, IL-6, TNF-α, MMP-8, and MMP-9 Levels in GCF

IL-1β, IL-6, TNF-α, MMP-8, and MMP-9 levels in GCF were identified using commercial ELISA methods with IL-1β, IL-6, TNF-α, MMP-8, and MMP-9 ELISA kits (Assaypro LLC & Angiopharm LLC, MO, USA, RayBiotech, Inc, GA, USA**)**. Stock standard within the kit was reconstituted according to the manufacturer's directives to prepare the standards. Processes were conducted according to ELISA kits. Microtitration plaque was read with a 450-nm plaque optical reader (Microplate Reader Biotek, VT, USA) and measured for absorbance. Levels of samples were multiplied with the reconstitution coefficient, 500, to reflect the concentration of IL-1β, IL-6, TNF-α, MMP-8, and MMP-9, and the concentration in GCF was calculated.

Statistical Evaluation

Data were evaluated using a computer-based statistical package program (SPSS 16.0). Definitive statistical data for each individual were recorded. While investigating the intergroup difference at each parameter, T test was used in independent groups. The level of significance was set at $p<0.05$.

RESULTS

No statistical difference was found between age groups and gender groups in the study ($p>0.05$). The mean age for systemically healthy-chronic periodontitis patients was identified as 42.12, and the range of age was 37-46. The mean age of patients with T2DM chronic periodontitis was 41.23, and range of age was 36-45 (Table 1).

	Chronic Periodontitis N: 25	T2DM-Chronic Periodontitis N: 25
Age (years)	42.12±4.24	41.23±5.38
Gender (female/male)	13/12	12/13

Table 1: Age and Gender Data for Patients Enrolled to Study (Mean±Standard deviation).
*Significantly different from other group, $p<0.05$

Periodontal clinical parameters (PI, GI, PD, BOP, and CAL) are given in Table 2. Periodontal clinical measurements were identified as statistically lower in systemically healthy-chronic periodontitis patients compared to patients with T2DM-chronic periodontitis ($p<0.05$).

	Chronic Periodontitis N: 25	T2DM-Chronic Periodontitis N: 25
GI	2.05±0.07	2.37±0.29*
PI	2.04±0.07	2.28±0.29*
PD (mm)	3.62±0.31	4.01±0.41*
CAL (mm)	3.50±0.29	3.84±0.42*
BOP%	75.60±8.72	82.80±10.41*

Table 2: Clinical Periodontal Parameters of Study Groups (Mean±Standard deviation).
PI; plaque index, GI; gingival index, PD; probing depth, CAL; clinical attachment loss, BOP; bleeding on probing.
*Significantly different from other group, $p<0.05$

IL-1β, IL-6, TNF-α, MMP-8, and MMP-9 levels in GCF for all groups are given in Table 3. IL-6, MMP-8, and MMP-9 levels in GCF were 0.235±0.01 ng/ml, 3580±590 pg/ml, and 2740±435 pg/ml, respectively, in patients in the

T2DM-chronic periodontitis group, and were 0.165±0.018 ng/ml, 1470±380 pg/ml, and 1350±634 pg/ml, respectively, in the systemically healthy-chronic periodontitis group (p<0.05). When IL-6, MMP-8, and MMP-9 levels were evaluated in GCF, the levels of IL-6, MMP-8, and MMP-9 in patients with T2DM-chronic periodontitis were higher than those of systemically healthy-chronic periodontitis patients. The difference was statistically significant (p<0.05). From the data evaluated in the study, IL-1β, and TNF-α levels were measured as 128±24 pg/ml and 0.612±0.033 ng/ml, respectively, for patients with T2DM chronic periodontitis and as 62±14 pg/ml and 0.435±0.021 ng/ml, respectively, for systemically healthy-chronic periodontitis patients. IL-1β and TNF-α levels in systemically healthy-chronic periodontitis patients were lower compared to patients with T2DM-chronic periodontitis, and the difference was statistically significant (p<0.05).

	Chronic Periodontitis N: 25	T2DM-Chronic Periodontitis N: 25
IL-1β (pg/ml)	62±14	128±24*
IL-6 (ng/ml)	0.165±0.018	0.235±0.01*
TNF-α (ng/ml)	0.435±0.021	0.612±0.033*
MMP-8 (pg/ml)	1470±380	3580±590*
MMP-9 (pg/ml)	1350±634	2740±435*

Table 3: GCF IL-1β, IL-6, TNF-α, MMP-8, and MMP-9 Levels of Study Groups (Mean±Standard deviation).
*Significantly different from other group, p<0.05

DISCUSSION

The duty of the host defense system is to protect against infectious agents. Skin and mucous membranes create physical barriers against microorganism attacks and toxins and enable host defense. The flushing impact of liquids such as saliva and GCF is removed from organisms that invade in mucosal surfaces, and enables protection with bactericidal agents. The strict epithelial barrier of gingival sulcular epithelium and connection epithelium is known to block the invasion of microorganisms and products in periodontal tissues. Besides being complements to GCF, saliva and serum act as elements of host defense.[18, 19] As a result of an immuno-inflammatory response developed in periodontal tissue that coincides to periodontal pathogen microorganisms, an increase occurs in the construction of inflammatory cytokines (IL-1β and TNF-α), chemotactic cytokines (IL-6), and tissue-destructive enzymes (MMPs). These proinflammatory mediators and enzymes are responsible for a great part of the destruction observed in periodontal disease.[20] The balance between inflammatory-anti-inflammatory cytokines and enzymes is more significant than the level of each inflammatory mediator found in periodontal tissues. The imbalance between cytokines and their inhibitors is the greatest factor responsible for the destruction of periodontal tissues.[21] Periodontal diseases may be defined as the inflammatory response of periodontal tissues against oral bacterial changes. Bacterial biofilm is very significant in gingival inflammation in periodontal tissues and periodontal tissue destruction. IL-1β and TNF-α are known to be cytokines that play a rather significant role in alveolar bone destruction.[22] Cytokines that play a significant role in periodontal diseases play a significant role in the initiation, regulation, and prolongation of natural immune response.[23] IL-1β and TNF-α cause vascular changes and also the migration of effector cells such as neutrophilia to periodontium. Thus, periodontal pathogens are suppressed and diminished. However, when the persistent nature of subgingival plaque combines with non-compliant cytokine response, the combination may cause inflammation and tissue destruction. The induction of primary mediators such as IL-1β and TNF-α stimulates the release of secondary mediators such as cyclooxygenase that cause the production of prostoglandins or chemokines acting as chemotactic cytokines. This enables inflammatory response in two routes, including the release of enzymes that cause collagen tissue destruction, and the resorption of osteoclastic bone resorption. Gelatinases (MMP-2 and MMP-9) act by destroying type IV collagen, laminin, and other basal membrane components. MMP-9 is an enzyme that allows insulin degradation. High levels of glucose contribute to the activation of latent MMP-9. Also, MMP-9 cells are considered to increase T-cell proliferation.[24] MMP-1, MMP-8, MMP-13, and MMP-18 are included in the collagenase group of enzymes. The basic property of these enzymes is the ability to destroy type I, II, and III collagens from a special region.[25] In our

study, IL-1β, IL-6, TNF-α, MMP-8, and MMP-9 levels in the GCF of patients with T2DM-chronic periodontitis were statistically higher than in systemically healthy-chronic periodontitis patients.

Vascular tissues of patients with DM were investigated and irreversible damage was observed in veins. In studies on DM patients, some changes were observed in factors produced and excreted from endothelium. This observed pathologic process may cause irreversible damage due to long-term hyperglicemia and changes in metabolic routes.[26, 27] A study conducted in 2007 by Navarro-Sanchez et al.[28] compared TNF-α and IL-1β levels in GCF for T2DM patients with chronic periodontitis and systemically healthy patients with chronic periodontitis, and no statistically significant difference was observed. However, following periodontal treatment, the levels of both cytokines in GCF were reduced in both groups. In a study conducted in 2007 by Engebretson et al.[29], IL-1β levels in GCF were higher in uncontrolled diabetic patients compared to systemically healthy patients. Uncontrolled diabetes increases xerostomia and sensitivity to oral infections including periodontitis.[30, 31] It is suggested that TNF-α and IL-1β produced as a result of periodontal infection in T1DM increase tissue resistance to insulin and are responsible for the hyperglycemic scheme.[32] The levels of IL-1β in periodontal inflamed tissues and GCF were increased in DM patients compared to healthy volunteers.[33,34] The change in monocyte/macrophase phenotype in DM causes an increase in proinflammatory cytokine, such as IL-1β and IL-6, and plays a significant role in the pathogenesis of periodontal diseases.[35-37] Duarte et al.[33] evaluated the cytokine levels of patients with DM-chronic periodontitis in gingival tissues, and reported that the increased expression of IL-1β and IL-6 was higher in patients with DM periodontal inflammation compared to patients with non-DM-chronic periodontitis ($p<0.05$). It is reported that the increased cytokine expression may cause a higher amount of periodontal destruction in DM patients. Studies revealed that MMP-9 expression increases in DM patients.[38, 39] It is also known to destroy the balance between MMP and its inhibitors, which are significant in wound-healing for patients with DM. It is suggested that excessive proteolysis may delay wound-healing in ulcers on the skin [40] and deeper periodontal pocket and CAL in diabetic patients.[41] In studies conducted on rats and humans, the predominant forms of MMP-2 and MMP-9 were defined, and it is reported that these MMPs are also related to the severity of periodontal disease and inflammatory response, as well as the regulation of cellular migration.[42, 43] Especially, MMP-9 is expressed from osteoclasts and degrades the collagens in the bone matrix and its related proteins.[44] While various MMPs that include MMP-2 may be produced from cells with healthy tissues, MMP-9 is secreted as a response to growth factors and cytokines from a limited number of cells.[45] This reveals that it can be a significant inflammatory mediator for the development of periodontal disease.[46]

In conclusion, irreversible vascular damage was observed in patients with DM. As a result of this damage, retinopathy, nephropathy, and neuropathy occur in these tissues. In addition to tissue damage, the destruction in periodontal tissues is more severe due to microorganisms and toxins in dental plaques when periodontal diseases are observed. Due to the vascular damage that accompanies the increase of destruction and changes in the microorganism profile, increased levels of IL-1β, IL-6, TNF-α, MMP-8, and MMP-9 were observed in GCF in patients with DM and periodontal disease. It is suggested that the identification of IL-1β, IL-6, TNF-α, MMP-8, and MMP-9 levels in GCF may enable the definition of pathogenesis in both disease groups and the explanation of potential methods to treat diseases. Further studies are required to completely explain the relationship between periodontal disease, DM, and IL-1β, IL-6, TNF-α, MMP-8, and MMP-9 levels.

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