



The Effect of the Overweight on the Circulating Cytokines and Adipokines in Experimental Periodontitis

DeneySEL Periodontitiste Fazla Kilolu Olma Durumunun Dolaşımdaki Sitokinler ve Adipokinler Üzerine Etkisi

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Abstract

Objective: Interleukin (IL)-1 β , IL-4, IL-6, and tumor necrosis factor (TNF- α) are forefront cytokines in the periodontitis pathogenesis. Therefore, this study aimed to test the hypothesis that overweight rats with periodontitis have higher pro-inflammatory cytokines and/or adipokines, and lower anti-inflammatory cytokines and/or adipokines in serum than normal weight rats.

Material-Method: Thirty-two 4-month old male Wistar rats were divided into 4 groups: normal weight, periodontally healthy rats (NH, n = 8), normal weight rats with periodontitis (NP, n = 8), overweight, periodontally healthy rats (OH, n = 8); and overweight rats with periodontitis (OP, n = 8). Periodontitis was induced by ligature for 14 days. The serum cytokine' and adipokine' levels were investigated using ELISA.

Results: IL-4 levels were higher in the NH and NP groups than their counterparts in the overweight rats (P < 0.01). The IL-6 level was significantly higher in the OP group than in the NP group (P < 0.01). The adiponectin level was significantly higher in the NH group than in the OH and OP groups (P < 0.01). Leptin and resistin levels didn't differ significantly among the groups (P > 0.01).

Conclusions: The overweight status affected the cytokine' and adipokine' level independent from the periodontal status in this experimental model. Overweight rats with and without periodontitis have lower serum levels of anti-inflammatory cytokine and adipokines (IL-4 and adiponectin) and higher serum levels of pro-inflammatory cytokine (IL-6) than their counterparts in normal weight rat groups.

Keywords: Periodontitis, Obesity, Overweight, Cytokines, Adipokines

Introduction

Periodontitis, an inflammatory disease accompanied by the destruction of bone and connective tissue and loss of teeth, is caused by dental plaque bacteria and their metabolic products and the host's immune response to these bacteria/bacterial

Özet

Amaç: İnterlökin (IL)-1 β , IL-4, IL-6 ve tümör nekrozis faktör (TNF)- α periodontal patogeneizde anahtar sitokinlerdir. Bu çalışma periodontitisi olan fazla kilolu ratlarda serumda daha yüksek düzeyde pro-inflamatuvar sitokin ve/veya adipokin ve daha düşük düzeyde anti-inflamatuvar sitokin ve/veya adipokin düzeyi olduğu hipotezini test etmeyi amaçlamaktadır.

Materyal-Metot: Dört aylık 32 erkek Wistar rat normal ağırlıklı ve periodontal sağlıklı (n=8), normal ağırlıklı periodontitisli (n=8), fazla kilolu periodontitisli (n=8) ve fazla kilolu periodontal sağlıklı (n=8) olmak üzere dört gruba ayrıldı. Periodontitis ligatürle 14 günde oluşturuldu. Serum sitokin ve adipokin düzeyleri ELISA ile incelendi.

Bulgular: Normal ağırlıklı periodontitisli olan ve olmayan ratlar fazla kilolu olan eş gruplarından daha yüksek IL-4 düzeyi sergiledi (P < 0,01). Fazla kilolu periodontitisli ratların IL-6 düzeyleri normal kilolu periodontitisli ratlardan anlamlı düzeyde yüksek bulundu (P < 0,01). Adiponektin düzeyi normal kilolu periodontal sağlıklı ratlarda fazla kilolu gruplardan daha yüksekti (P < 0,01). Leptin ve rezistin düzeyleri gruplar arasında farklı bulunmadı (P > 0,01).

Sonuç: Bu deneysel modelde fazla kilolu olma durumu serum sitokin ve adipokin düzeylerini periodontitisten bağımsız olarak etkilemiştir. Fazla kilolu olan periodontitis olan ve olmayan ratların anti-inflamatuvar sitokin ve adipokinler (IL-4 ve adiponektin) düşük, pro-inflamatuvar bir sitokin olan IL-6 ise normal ağırlıklı eşlerinden daha yüksek olarak bulunmuştur.

Anahtar Kelimeler: Periodontitis, Obezite, Fazla Kilo, Sitokinler, Adipokinler

products (1). Most current research is focused on the balance/ imbalance of immunomodulatory molecules (cytokines, adipokines, growth factors, hormones, and chemokines) (2). The nature (pro- or anti-inflammatory) of the cytokines and mediators, their relationships, and balance with each other

affect the amplitude of the inflammation and the amount of associated tissue destruction (3).

Overweight and obesity are worldwide health problems predisposing major chronic diseases including cardiovascular disease and diabetes mellitus (4, 5). Obesity has also been identified as the second most-important risk factor, following smoking, for periodontal disease (6). It was recently reported that overweight/obesity and longitudinal weight changes result in deterioration of the periodontal pocket depth and, alveolar bone, as well as attachment loss (7). Although the pathway between overweight/obesity and periodontitis has not been clarified, some mechanisms linking periodontitis to overweight/obesity have been suggested. The accumulation of fat was reported to lead to low-grade systemic inflammation (4). Besides, obesity and related fat accumulation might be responsible for the release of local and systemic pro-inflammatory cytokines and adipocytokines (including leptin, adiponectin and resistin) from adipocytes, resulting in modifications of the host response-, and playing a role in periodontal hard- and soft- tissue destruction (8-10).

The studies have shown the causal relationship between the cytokines and tissue destruction (11). Interleukin (IL)-1, IL-4, IL-6 and tumour necrosis factor (TNF)- α were determined as key cytokines; IL-1, IL-6 and TNF- α have pro-inflammatory, IL-4 has anti-inflammatory roles in periodontal pathogenesis (12). All of these and other mediators have associations between each other via different pathways, targeting to prevent, stop or limit the tissue destruction. Thus, the key cytokines have “communications” with each other in the inflammatory process of periodontal disease (13).

The adipokines, other immunomodulatory mediators, including, leptin, adiponectin and resistin, are bioactive molecules produced by adipose tissue (14). Adipose tissue has the potential to produce the cytokines mentioned above, IL-1 β , IL-6 and TNF- α , and increase their levels in systemic circulation (15-17). Leptin and resistin were reported having pro-inflammatory, and adiponectin was reported to have anti-inflammatory functions in inflammation (14, 15, 18).

The role of pro- and anti-inflammatory cytokines and adipokines has not been clarified in the relationship between overweight and periodontitis yet. Thus, the mechanism(s) need to be clarified in order to take measures to prevent new periodontitis entities and to develop new treatment alternatives for the present periodontitis cases regarding host modulation.

Therefore, this study aimed to test the hypothesis that overweight rats with periodontitis have higher pro-inflammatory cytokines and/or adipokines, and lower anti-inflammatory cytokines and/or adipokines in serum than normal weight rats from the same generation.

Material-Methods

Ethical approval was received from the Süleyman Demirel University Local Ethical Committee on Animal Experiments (date: 2010, decision number: 02). All experimental procedures were performed at the Süleyman Demirel University Laboratory of Experimental Animals Research

Center. The rats were housed under the same optimized environmental conditions, at a constant room temperature of 21°C and humidity of 50–60%, with a 12:12-hour light-dark cycle (light on at 0700 hours). The rats were housed 4 to a cage, were fed standard S2 pellets ad libitum (Zirve, İstanbul, Turkey), also had access to tap water ad libitum.

Experimental Design

Sample size estimate was based on the study of Yan and Wei (19) considering alveolar bone loss. Thus, 32 4-month-old male albino Wistar rats from the same generation, weighing between 180 and 329 g, were used. The study comprised the following 4 groups of rats from the same generation and same environmental housing conditions: normal weight periodontally healthy rats (NH, n=8), normal weight rats with periodontitis (NP, n=8), overweight periodontally healthy rats (OH, n=8), and overweight rats with periodontitis (OP, n=8).

The Induction of Periodontitis

Experimental periodontitis was induced using the ligature method. The rats were anaesthetized using an intraperitoneal injection of ketamine HCL (0.1 ml/100 g, Pfizer, İstanbul, Turkey) and xylazine HCL (0.05 ml/100 g, Biopharm, İstanbul, Turkey). The ligatures (3.0 silk sutures, Doğsan, İstanbul, Turkey) were placed bilaterally at the upper second molars, and the knots were placed on the cervico-palatal surfaces of the molars (20). After the development of periodontitis (14 days), the rats were sacrificed by exsanguination from the aorta after anaesthetized. The rats were decapitated, the maxillae excised, the soft tissues removed, and the maxillae separated into halves. All of the isolated maxillary segments were left in H₂O₂ (3%, +4 °C) for 24 h and then fixed in formalin (10%, +4 °C). The periodontally healthy rats (NH and OH) were sacrificed after 14 days without any intervention. The same procedures were performed after sacrifice.

Determination of the Presence of Periodontitis

A pre-experimental examination was performed to exclude animals with periodontal probing depths exceeding 0.5 mm (21) to ensure that animals were periodontally disease-free before the induction of experimental periodontitis. All of the ligatured teeth exhibited periodontitis after the experiment. Stereomicroscopic evaluation (Olympus SZ-PT, Tokyo, Japan) confirmed these observations; the maxillae were photographed (Olympus C-4000 ZOOM, Tokyo, Japan) under the stereomicroscope and are shown in Figure 1A. The present study employed the direct digital radiography method (22, 23; Figure 1B). The stereomicroscopic and digital radiographic evaluations were conducted only to evidence the periodontal breakdown. The alveolar bone loss was not measured.

Biochemical Analysis

A total of 5-7 mL blood was collected from each rat after exsanguination. The serum samples were separated by centrifugation and portioned into Eppendorf tubes and stored at – 80 °C until the analysis. The presence and levels of interleukin (IL)-1 β , IL-4, and IL-6 (Bender MedSystems, Vienna, Austria), resistin and tumor necrosis factor (TNF)- α

(Assaypro, Missouri, USA), leptin (Biovendor, Modrice, Czech Republic), and adiponectin (Cusabio Biotech, Wuhan, China) were determined by using commercial ELISA kits. Standards and control serums were run in duplicate in each assay.

Statistical Analysis

The parameters are presented as the median (minimum–maximum range). The Kruskal-Wallis test was used to determine the between-group differences. A P value of less than 0.05 was considered significant. To avoid type-I errors, the Bonferroni correction was applied, and the differences between the group pairs were investigated using the Mann-Whitney U test, with a P value of less than 0.01 being considered significant. Correlations between serum parameters were analysed using Spearman and Pearson correlation tests. Statistical analysis was performed using statistical software (SPSS 15.0, Chicago, IL, USA).

Results

Periodontitis developed over 14 days in all of the rats, without complications and animal loss in any group. The rats did not show any obvious signs of systemic illness throughout the study period, regardless of group.

The Body Weight Changes of the Study Groups

At the baseline, significant differences between the groups NH and OH; NH and OP, NP and OH, and NP and OP, were found ($P < 0.01$, Table 1). After 14 days, significant differences were found between the groups NH and OP, NH and OH, NP and OP, NP and OH, ($P < 0.01$, Table 1).

The Serum Levels of Cytokines and Adipokines

The concentrations of the serum parameters and the significant differences between the groups are presented in Table 2.

Table 2. The serum concentrations of the cytokines and adipokines [median (minimum- maximum)]

Parameters	NH (n=8)	NP (n=8)	OH (n=8)	OP (n=8)	P
IL-1 β (ng/ml)	31.10 (11.40 - 207.20)	75.50 (11.40 - 400)	11.20 (10 - 258.60)	53.95 (11.40 - 232)	>0.05
IL-4 (ng/ml)	5.36 (2.78 - 6.12)	4.11 (3.68 - 5)	2.86 (2.10 - 4)	3.46 (2.78 - 5.82)	0.004 ^b 0.003 ^c
IL-6 (ng/ml)	3.18 (1.60 - 6.27)	13.92 (7.54- 23)	26.07 (13.00 - 42.01)	26.5 (12 - 41)	0.001 ^a 0.001 ^b 0.004 ^c 0.001 ^d
TNF- α (ng/ml)	0.17 (0.01 - 0.90)	0.56 (0.02 - 1.2)	0.82 (0.04 - 1.66)	0.79 (0.28 - 2)	>0.05
Leptin (ng/ml)	3.64 (2.90 - 6.11)	3.66 (2.58 - 6.59)	5.07 (3.28 - 6.97)	4.20 (3.23 - 6.97)	>0.05
Adiponectin (ng/ml)	6.95 (4.35 - 7.60)	6.30 (1.04 - 8.49)	4.90 (0.90 - 6.34)	3.33 (0.70 - 4.64)	0.005 ^b 0.007 ^c 0.001 ^d
Resistin (ng/ml)	8.46 (0.86 - 36.85)	6.41 (0.60 - 56.52)	6.13 (0.60 - 32.88)	12.88 (0.60 - 94.73)	>0.05

NH: normal weight periodontally healthy rats, NP: normal weight rats with periodontitis, OH: overweight periodontally healthy rats, OP: overweight rats with periodontitis, a: statistically significant difference between NH and NP groups, b: statistically significant difference between NH and OH groups, c: statistically significant difference between NP and OP, d: statistically significant difference between NH and OP groups ($P < 0.01$).

The serum IL-1 β and TNF- α levels were not statistically significantly different among the study groups ($P > 0.05$, Table 2). Serum IL-4 levels were higher in the NH group than in the OH group, and as well in the NP group than in the OP group ($P < 0.01$, Table 2). The serum concentration of IL-6 was higher in the NP, OH, and OP groups than in the NH group and higher in the OP group than in the NP group ($P < 0.01$, Table 2). The IL-4 and IL-6 levels did not differ significantly between the overweight (OH and OP) subgroups ($P > 0.01$, Table 2). The adiponectin level was significantly higher in the NH group than in the OH and OP groups and in the NP group than in the OP group ($P < 0.01$, Table 2). The leptin and resistin levels were not statistically significantly different among the study groups ($P > 0.05$, Table 2).

Table 1. The definitive statistics of the rats regarding to their body weights on baseline and day 14 [median (minimum- maximum)]

Groups	Body Weight	
	Baseline	Day 14
NH	184 (180-190) c,d	184 (180-190) c,d
NP	184 (180-192) c,d	198.50 (192-216) c,d
OH	296.50 (279-329) a,b	310 (285-333) a,b
OP	309 (270-329) a,b	312.50 (284-330) a,b

NH: normal weight periodontally healthy rats, NP: normal weight rats with periodontitis, OH: overweight periodontally healthy rats, OP: overweight rats with periodontitis, a: significantly different than NH group, b: significantly different than NP group, c: significantly different than OH group, d: significantly different than OP group, ($P < 0.01$).

Table 2. The serum concentrations of the cytokines and adipokines [median (minimum- maximum)]

Groups	Parameters	r	P
Whole group (NH+NP+OH+OP, n=32)	Leptin - TNF- α	0.606	<0.001
	Adiponectin - IL-4	0.506	0.003
	Adiponectin - IL-6	-0.464	0.007
	TNF- α - IL-6	0.502	0.003
	IL-4 - IL-6	-0.437	0.012
Periodontally healthy (N +O, n=16)		rho	
	Leptin - TNF- α	0.671	0.004
	Adiponectin - IL-4	0.620	0.010
	Adiponectin - TNF- α	-0.682	0.004
	Adiponectin -IL-6	-0.604	0.013
	IL-1 β - IL-6	-0.550	0.027
	IL-4 - TNF- α	-0.556	0.025
	IL-4 - IL-6	-0.523	0.038
Periodontitis (N +O, n=16)	Leptin - TNF- α	0.506	0.046
	Adiponectin - IL-4	0.506	0.046
	Resistin - IL-1 β	-0.554	0.026
Normal weight (H+P, n=16)	Leptin - TNF- α	0.541	0.030
Overweight (H+P, n=16)	Leptin - Adiponectin	0.505	0.046

NH: normal weight periodontally healthy rats, NP: normal weight rats with periodontitis, OH: overweight periodontally healthy rats, OP: overweight rats with periodontitis, N: normal weight rats with and without periodontitis, O: overweight rats with and without periodontitis, H: normal weight and overweight rats with healthy periodontium, P: normal weight and overweight rats with periodontitis, r: Pearson correlation coefficient, rho: Spearman's correlation coefficient

Correlations Between the Serum Parameters

The correlations between the serum parameters were evaluated for the whole group (n = 32), regarding body weight (including both rats with healthy periodontium and those with ligature-induced periodontitis, n = 16 each group) and regarding periodontal health (including both normal weight and overweight rats, n = 16 each group). Only the statistically significant correlations are presented (Table 3).

Discussion

This study investigated whether overweight rats have higher pro-inflammatory cytokines and/or adipokines, and lower anti-inflammatory cytokines and/or adipokines in serum than normal-weight rats from the same generation.

Actually, the overweight rats might be defined as –obese- because of a greater than 15% difference between the groups according to Svensson et al. (24) and Verzeletti et al. (25). In the present study, the body weight differences at the baseline were 38% between the NH and OH groups and, 41% between the NP and OP groups. However, in the literature, the studies have presented genetically obese animals, or have induced obesity by high- calorie/high- fat diets (26-28). It is also noteworthy, that the studies in the literature used rats in a large body weight spectrum between 277 and 630 g, determining as “obese”. The classification of rats as –obese- should be based on Lee index (29) or body mass index (BMI) values (30). In the present study, we eliminated the effect of high- calorie/ high- fat diets on increasing the body weight and the related generation of hyperglycemia, hyperinsulinemia, and glucose intolerance (30, 31); in addition no movement restriction was applied. We have defined the rats with higher body weight – overweight- instead of –obese-.

The starting point of our study was to test the possible differences of systemic cytokine'/adipokine' levels to clarify the mechanism between overweight status and periodontitis. For this purpose, the serum levels of IL-1 β , IL-4, IL-6, and TNF- α were evaluated. The chronic inflammatory lesion of periodontitis is characterized by the increase of IL-1 β and TNF- α (32). The anti-inflammatory cytokine IL-4 inhibits the production of IL-1 β , TNF- α and IL-6 (32, 33). The local absence of IL-4 has been reported to be associated with the transformation of gingivitis to periodontitis and the progression of periodontitis (34). The IL-4 levels were found to be higher in the NH rats than in the OH rats. The lower level of IL-4 in the OP and OH groups than their counterparts of normal-weight rats might be the result of systemic inflammation related to immunomodulation of the overweight status. The serum levels of the cytokines (IL-1 β , IL-4, and TNF- α) were not influenced by the presence of periodontitis and related inflammation. The study duration was two weeks (35); however, further studies should investigate these cytokines and adipokines over a longer study period (36). To investigate the local levels of cytokines/adipokines regarding protein level and gene expressions in the gingiva might give more detailed results. In addition, the local evaluations should include the alveolar bone loss measurements.

Adipokines and related low-grade systemic inflammation
Adipokines and related low-grade systemic inflammation were previously proposed to be one of the mechanistic links between periodontitis and overweight/obesity. In the present study, the forefront adipokines were evaluated. Leptin was previously reported as pro-inflammatory based on higher serum levels in periodontitis patients (37-39). However, Yetkin Ay et al. (40) did not find significant differences between the gingivitis, chronic and aggressive periodontitis,

and control groups regarding serum leptin levels. These discrepancies between the studies might be the result of the different methodology. In the present study, the similar serum leptin levels might be the result of the study duration and the body weight of the rats. However, the positive correlations between leptin and TNF- α denoted the pro-inflammatory feature of leptin.

In the present study, all of the groups demonstrated similar serum resistin levels. Patel and Raju (41) reported increased serum and gingival crevicular fluid levels in obese periodontitis patients when compared to non-obese periodontitis patients and controls, similar to the results of Saito et al. (42) and Hiroshima et al. (43). This discrepancy with the human studies might result from the different primary origins and regulatory mechanisms of resistin in humans and rodents. Another reason might be the body weight, amount of visceral fat (44), and the degree of obesity (45).

In our study, serum adiponectin levels were significantly higher in the NH group than in the OH and OP groups. The presence of periodontitis does not influence the serum adiponectin level. Besides, the positive correlation with IL-4 and the negative correlations with IL-1 β and TNF- α are in concordance with classification as -anti-inflammatory-. Our results regarding lower adiponectin levels in overweight rat groups are also concordant with the report that lower adiponectin levels increase the risk for periodontal pocket formation in obese subjects (46,47).

In the present study, the hypothesis that overweight rats with periodontitis would present higher pro-inflammatory cytokine' and adipokine' levels in circulation was partially approved. The lower anti-inflammatory cytokine and adipokine levels (IL-4 and adiponectin) in serum of overweight rats with/without periodontitis than their normal weight counterparts, and the higher pro-inflammatory cytokine levels (IL-6) in serum of overweight rats with/without periodontitis than their normal weight counterparts was in accordance with the hypothesis of the present study. The differences regarding, IL-1 β , TNF- α , leptin and resistin were not found significantly different among the groups. That is the reason, why the hypothesis of our study was partially approved.

Our results are in concordance with the study by Mendoza-Azpur et al. (48), who reported that the adipokine' levels were not affected by the presence of chronic periodontitis between obese and normal-weight subjects. In contrast, Zimmermann et al. (49) found that lower systemic levels of adiponectin and higher systemic levels of resistin were associated with periodontitis. The higher systemic level of leptin in that study assumed to be the result of periodontitis and obesity (42).

Conclusion

The main limitation of our study is the absence of alveolar bone loss measurements. In case of the presence of this measurement the comparisons and correlations between the serum parameters among the groups would be more valuable and powerful.

Our study revealed important results concerning cytokines

and adipokines in periodontal pathogenesis and in coexisting periodontitis and overweight. In further studies, the rat groups might be constituted into normal-weight, overweight and obese groups. Thereby, the stages from normal weight to obesity might be evaluated gradually without overlooking the effect of overweightness on periodontal pathogenesis. Thus, the longitudinal changes of weight and adiposity, the fat per body (muscle quantity and fat distribution) might also be investigated. Although we did not investigate it, the lipid profile and the presence of any coronary artery disease should also be evaluated. Besides, our results should be evaluated with the consideration that the periodontitis and overweight models used were chosen to represent as closely as possible the periodontitis and overweight in human patients.

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