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Serum leptin levels and their proportional relationship with pro- and antiinflammatory mediators in aggressive periodontitis: preliminary report

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Aim: To investigate the serum level of leptin (OB) and its relationship with pro- and antiinflammatory cytokines/mediators in aggressive periodontitis (AP) patients.

Materials and methods: The study population (n = 12) comprised 6 AP patients and 6 periodontally healthy subjects (HC). The fasting venous serum samples were used to determine the OB and the cytokines/mediators belonging to the Th1/Th2/Th17 cells. The correlations among the OB and cytokines/mediators in the serum and clinical periodontal parameters were also investigated.

Results: The AP group presented significantly higher serum TGF-β1 and IL-2 concentrations (pg/mL) and lower IL-4, IL-5, IL-12, IL-13, and IL-17A concentrations than the HC group ($P < 0.05$). The ratios of OB to IL-12, IL-13, and IL-17A were lower and the ratio of OB to TGF-β1 was higher in the AP than in the HC group ($P < 0.05$).

Conclusion: Within the limitation of the small sample size, the results of our study are important in having determined the proportional relationship of OB with the pro- and antiinflammatory cytokines'/mediators' panel in AP patients. The usage of these kits in analyzing multiple cytokines/mediators might proportionally clarify the pathogenesis of AP.

Key words: Aggressive periodontitis, leptin, cytokines

1. Introduction

The cytokines affect various cells and play important roles in various inflammatory diseases such as periodontitis (1). They have roles in the function and differentiation of T cells, and they designate the T-helper subsets to amplify or control the amplitude of the inflammation and the destruction related to inflammation (2,3). The macrophages and T cells are dominate in the early/stable lesions of chronic periodontitis (CP), leading to the consideration that this response is developed by the Th1 cytokines. In the advanced/progressive lesions of CP, B cells and plasma cells are dominate and present the Th2 cytokines (1,2). Intriguingly, aggressive periodontitis (AP) was not the focus of the studies investigating the dominance of Th cell types. However, supporting the information above, it was suggested that AP lesions should present the predomination of Th2 because of its B cell-plasma cell nature (4).

It was suggested that the above-mentioned Th1/Th2 model could not be adequate to explain the complex interactions between the cytokines and immune cells (5). Recently, Th17 cells gained considerable attention with their cytokine profiles. In the Th17 cell development and IL-17 production, transforming growth factor (TGF)-β and IL-23 are determinants; it was revealed that the Th1 response could be decreased by the presence of IL-23 (6). Moreover, IL-12 or IL-4 can influence the Th17 cells to convert Th1 or Th2 cells (7).

Growth factors, hormones, and chemokines are the other immunoregulatory molecules that regulate the development and progression of periodontal diseases. Leptin (OB) is one of these immunomodulatory molecules. OB is one of the adipokines that have an effect on food intake and energy consumption. It is a 16-kDa nonglycosylated peptide, similar to those in the IL-6 family (8). Furthermore, it has been reported that OB has an immunomodulatory effect (9). The host response was mediated by OB in a proinflammatory way, activating the proinflammatory cells to produce proinflammatory cytokines (10,11).

In our previous study, OB and soluble OB receptors (s-OB-Rs) were investigated in the gingiva and in the

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serum of AP patients. The presence of OB and s-OB-R in the gingiva supported our hypothesis that these molecules may have a regulatory role in periodontal diseases and in AP pathogenesis (12). We suggested in our previous study, in light of our results, that further studies were needed in terms of the ratio of OB to the key cytokines, evidenced to play major roles in the periodontal disease pathogenesis, to possibly clarify their role in periodontal disease pathogenesis.

Similar to CP, in AP it is logical to investigate the balance, ratio, and relationship between the cytokine and immunomodulatory molecules (growth factors, hormones, chemokines, etc.) to clarify the etiology and pathogenesis of AP, since the pro- and antiinflammatory cytokines and other immunomodulatory molecules are produced and secreted by the same immune cells as in CP. In the present study, we investigated the proportional relationship of OB and key cytokines/mediators in a group of AP patients and compared them to a healthy control group (HC) using a multiple cytokine analysis kit.

2. Materials and methods

This study was conducted according to the ethical standards of the Declaration of Helsinki (Version VI, 2002). The details of study were explained and written consent was obtained from the subjects. In addition, the Ethics Committee of the Süleyman Demirel University Faculty of Medicine approved the study (6068, 22.12.2005). The volunteers were selected from subjects referred to the Süleyman Demirel University Faculty of Dentistry and Department of Periodontology between December 2005 and December 2008. The study population consisted of individuals from our previous study (12). Both of the groups (AP and HC groups) consisted of the first 6 people who participated the previous study. The diagnosis of the periodontal status was made regarding the criteria reported in the Consensus Classification of Periodontal Diseases (13).

2.1. Study population

Patients having systemic diseases (diabetes mellitus, cardiovascular disease, respiratory system diseases, osteoporosis, etc.), using drugs (antidepressants, antiinflammatory, antidiabetic, antihyperlipidemic, etc.) routinely, using antibiotics (in the last 3 months), or receiving periodontal treatment (in the last 6 months) were excluded from the study. The pregnant and lactating female subjects were also excluded. All of the subjects who participated in this study were nonsmokers.

2.2. Periodontal examination and recordings

The periodontal recordings, including gingival index (GI) (14), plaque index (PI) (15), bleeding on probing (BOP) (16), and probing depth (PD) and clinical attachment level (CAL) values, were recorded by a single examiner (ZYA) with 85% accuracy of measurements.

2.3. Biochemical analysis

The fasting blood samples (8 mL) were obtained in the morning. The blood samples were centrifuged (5 min at 5000 rpm), and the separated serum was portioned. The serum samples were stored at –80 °C until the analysis. Commercial ELISA kits were used to evaluate the levels of OB and the cytokine/mediator in serum samples (Biovendor, Czech Republic; Multi-Analyte ELISArray, SABioscience, USA).

2.4. Statistical analysis

Continuous variables were presented as median (minimum–maximum), and the categorical variables were presented as frequencies. The Mann–Whitney U test was used to analyze the differences between 2 groups ($P < 0.05$). The correlations between parameters were evaluated using the Spearman rho correlation coefficient ($P < 0.05$). The analyses were made using SPSS 9.0 (SPSS Inc., USA).

3. Results

Both of the groups consisted of 6 people (4 females, 2 males in the AP group; 3 females, 3 males in the HC group). The age ranges were 35–43 years (mean: 38.67 ± 3.50) in the AP group and 26–38 years (mean: 32.33 ± 4.13) in the HC group. The BMI values of the groups were as follows: 23.20 (20.70–34.30) in AP and 25.78 (19.40–34.60) in HC. The age and BMI values were not found to be significantly different between the groups ($P < 0.05$).

3.1. Clinical parameters

All of the clinical parameters were significantly higher in the AP group than the HC group ($P < 0.05$, Table 1).

3.2. Serum OB levels

The serum OB levels were 17.20 (8.49–43.30) in the AP group and 12.67 (6.76–22.36) in the HC group (pg/mL, median, minimum–maximum), and were not statistically significantly different ($P > 0.05$).

3.3. Cytokine and mediator levels and leptin/cytokine (mediator) ratios

In the AP group, the serum TGF-β1 and IL-2 concentrations (pg/mL) were significantly higher and the IL-4, IL-5, IL-12, IL-13, and IL-17A concentrations were significantly lower than in the healthy control group ($P < 0.05$, Figure). The OB/cytokine (mediator) ratios presented significant differences between the groups, as shown in Table 2. Briefly, the ratios of leptin to IL-12, IL-13, and IL-17A were lower and that to TGF-β1 was higher in the AP than the HC group ($P < 0.05$, Table 2).

3.4. Correlations

The statistically significant correlations between the parameters are presented in Table 3. All of the clinical parameters showed positive correlations with IL-12 ($P < 0.05$) and negative correlations with IL-17a and TGF-β1 ($P < 0.05$). The BMI values did not present any significant correlations with clinical and serum parameters ($P > 0.05$).

Table 1. The periodontal characteristics.

Groups/parameters	AP (n = 6)	HC (n = 6)	P
GI	1.85 (1.07–2.44)	0.22 (0–0.39)	*
PI	1.89 (1.26–2.67)	0.37 (0–0.87)	*
BOP (%)	93.65 (61.90–100)	13.98 (0–30.43)	*
PD (mm)	4.32 (3.55–5.18)	1.52 (1.41–2.27)	*
CAL (mm)	5.89 (5.33–6.81)	1.82 (0–2.27)	*

*: Significantly different (Mann–Whitney U test, $P < 0.05$).

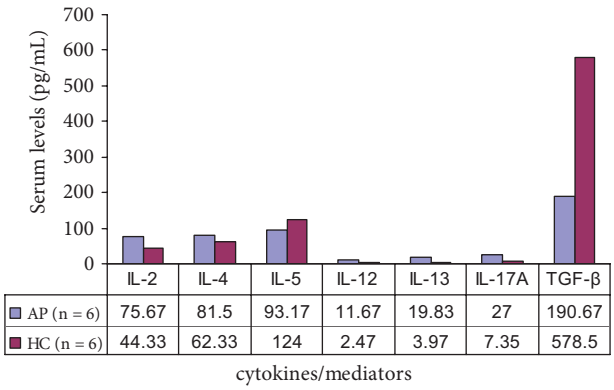

Figure. The serum cytokine and mediator levels (median) in the groups.

Table 2. The statistically significantly different OB/cytokine (mediator) ratios.

	AP (n = 6)	HC (n = 6)	P
OB/IL-12	1.57 (0.61–4.33)	5.23 (2.33–9.35)	*
OB/IL-13	0.92 (0.39–2.41)	3.25 (1.42–5.88)	*
OB/IL-17A	0.68 (0.27–1.88)	1.75 (0.79–2.98)	*
OB/TGF-β	0.10 (0.04–0.28)	0.02 (0.01–0.04)	*

*: Significantly different (Mann–Whitney U test, $P < 0.05$).

4. Discussion

To our knowledge, this is the first study that evaluated the serum OB levels proportionally to multiple cytokines/mediators in AP patients. These assays could be used in future studies to achieve a rough perspective about the relationship between the pro- and antiinflammatory cytokines/mediators in the pathogenesis of CP and AP. These assays could also be used to clarify the transition from the healthy state to gingivitis and from gingivitis to periodontitis. The individuals at risk for periodontitis, the presence of a subclinical disease, the diagnosis, the determination of disease activity, and the response to the treatment and the selection of therapies might be determined by the usage of such a biomarker panel.

Table 3. The significant correlations.

Parameters	*rho*	P
IL-12 - TGF-β	–0.767 **	0.004
BOP - G-CSF	–0.614 *	0.033
GI - TGF-β	–0.636 *	0.026
PI - TGF-β	–0.683 *	0.014
BOP - TGF-β	–0.827 **	0.001
PD - TGF-β	–0.692 *	0.013
CAL - TGF-β	–0.720 **	0.008
GI - IL-12	0.886 **	0.000
PI - IL-12	0.775 **	0.003
BOP - IL-12	0.726 **	0.007
PD - IL-12	0.764 **	0.004
CAL - IL-12	0.781 **	0.003
GI - OB/IL-17A	–0.818 **	0.001
PI - OB/IL-17A	–0.848 **	0.000
BOP - OB/IL-17A	–0.638 *	0.026
PD - OB/IL-17A	–0.594 *	0.042
CAL - OB/IL-17A	–0.664 *	0.018
GI - OB/IL-2	–0.580 *	0.048
BOP - OB/IL-12	–0.674 *	0.016
PD - OB/IL-12	–0.636 *	0.026
CAL - OB/IL-12	–0.678 *	0.015
BOP - OB/TGF-β	0.725 **	0.008
PD - OB/TGF-β	0.762 **	0.004
CAL - OB/TGF-β	0.706 *	0.010

Spearman's correlation test (*: $P < 0.05$, **: $P < 0.01$).

Another important advantage of these kits is the relatively ideal working conditions. Ideally, for reliable results the same conditions are preferred. For example, all of the kits (12 different ELISA kits for 12 different serum parameters) are preferred to be used at the same time of day, under the same laboratory conditions (at the same temperature and humidity), and by the same person. These conditions (same person, same time interval, same temperature, etc.) could not be achieved in our studies when investigating more than 3 parameters (cytokines/mediators). In addition to having the advantages mentioned above, these assays used in the present study are easy to use and with their low costs they are candidates to be more expansive. However, we agree with Thunell et al. (17), who stated that it was important to consider interassay disparities in terms of the particular technique that was used (i.e. multiplex bead assays or ELISA kits) used and possible interlaboratory variations. It should also be kept in mind that it would be logical not to compare the results that belong to one assay to another assay used in different studies (18).

With this preliminary study, we have obtained a general perspective about the proportional balance between OB and cytokines/mediators. Although the very limited study sample would not give the opportunity to make clear comments about this issue, some results were intriguing. In the present study, the serum OB levels were similar in both of the groups, and the ratios of OB to some cytokines/mediators (IL-12, IL-13, IL-17A, and TGF-β1) were found to be significantly different. These significant differences depend on the significantly different concentrations of these cytokines and growth factor between the groups. Despite the insignificantly different serum OB levels in the AP and HC groups, we speculate that an "alternate leptin pathway" exists, as we suggested in our previous study (12). This "alternate leptin pathway" might be regulated by these above-mentioned factors (IL-12, IL-13, IL-17A) and by other cytokines.

By the usage of the multiple cytokine/mediator analysis kit in this study, the Th1, Th2, and Th17 cells and their cytokine/mediator productions are dependent upon each other with various routes in periodontitis, and also in AP patients. In addition, the correlations between the clinical parameters and IL-12, IL-17, and TGF-β have led us to consider that there is an imbalance towards the Th2 type of reaction in AP to avoid the destruction directed toward the host tissues.

The Th1/Th2 balance was reported to be regulated by IL-12 in favor of the Th1-specific manner, with concomitant production of interferon (IFN)-γ, IL-1β, tumor necrosis factor (TNF)-α, and IL-8 from T cells and natural killer cells. Thus, the early innate immune response shifted to the adaptive immune response (19).

The role of IL-13 in periodontitis was not clarified. In periodontitis lesions, the expression of IL-13 was demonstrated (19,20). IL-4 and IL-13 have effects on fibroblasts by activating TGF-β production in macrophages (21). The localized absence of IL-4 in periodontally diseased tissues was reported to be related to the periodontal disease activity and progression (22). In the present study, the serum IL-4 and IL-13 levels were higher in AP patients than in the HC group, which led to the consideration that the AP lesions have a balance towards Th2 reaction because of the B cells' predominance.

It was previously reported that IL-4 might be classified as a B-cell growth factor (23).

The serum level of IL-17 of AP patients was found higher than that of the HC group in the present study. The proinflammatory nature of IL-17 was also reported (24,25). TGF-β and IL-6 presences have modulated the naive T cells to differentiate to Th17 (5). IL-12 or IL-4 has been reported to affect the TH17 cells to differentiate to Th1 or Th2 cells (6). IL-23 regulates the Th1 differentiation from Th17 negatively (5). Unfortunately, the cytokine/mediator panel used in our study did not include IL-23 to enhance our comments on this regulatory role of IL-23 on the Th1 response inhibited by IL-17.

In the present study, the serum level of TGF-β in the AP group was lower than in the HC group. The functional properties of TGF-β were determined and reported as cell growth regulation, differentiation and matrix production, and remodeling in both soft and mineralized connective tissues (26,27).

The roles and functions of the cytokines and mediators, which were significantly different between the serum samples of AP and HC subjects, were concluded above. In this regard, it is obvious that a balance between Th1/Th2 and Th17 cells and cytokines in periodontal disease exists. It is necessary to investigate this balance with its components (cytokines/mediators and cells) in the pathogenesis of AP. The studies should focus on these interactions, and accordingly on the balance and imbalance between these cells types and their productions, the key mediators regulating the events in inflammation in the initiation and progression, not only in CP but also in AP. The results obtained from these studies allow researchers to develop new treatment strategies based on the findings, such as immunomodulatory therapies. Larger study groups are required to investigate the levels of OB and Th1/Th2/Th17 cytokines/mediators in gingival crevicular fluid, serum, and gingival tissues of AP patients.

Conclusively, within the limited sample size of the present study, OB did not present a significant difference between the groups. When the levels of serum OB in the AP (17.20 pg/mL) and in the HC (12.67 pg/mL) groups are taken into consideration, it might be speculated that the difference might be significant if the sample size were larger. However, the presence of leptin reported in the gingiva of the AP patients in our previous study (12) led us to consider that OB levels might have an effect on periodontal inflammation of AP patients and might be regulated by the cytokines/mediators, standing on the forefront in the results of the present study. In further studies, the study population should be subgrouped not only according to the periodontal status (healthy and AP), but also to systemic status, and especially the presence of diabetes (the levels of some cytokines/mediators were found to be significantly different in diabetic subjects than in healthy subjects) (28,29)). The study population should also be organized according to the serum lipid profiles, to the different BMI values, and the body fat mass and hip/wrist ratio, which may provide more accurate information about the role of OB in AP pathogenesis.

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