Correlation between TNFα in gingival crevicular fluid and body mass index in obese subjects

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The aim of this study was to investigate the relationship between body mass index (BMI kg/m^2), the inflammatory mediator tumor necrosis factor α (TNFα), and interleukin-8 (IL-8) in gingival crevicular fluid (GCF) from 32 obese subjects aged between 13 and 24 years. Gingival inflammation (GBI %), pathological pocket depths, and alveolar bone loss diagnosed on radiographs were recorded. The GCF was collected from six sites per subject using periopaper, and the volume was determined using Peritron 8000. The levels of TNFα and IL-8 were determined using ELISA kits. Within the whole group, there was no significant relationship between BMI and the variables age, GBI %, number of periodontal pockets, smoking, and the levels of TNFα or IL-8. In subjects with BMI ≥40, however, there was a statistically significant correlation ($r = 0.74$, $P < 0.01$) between the level of TNFα in GCF and BMI. The correlation coefficient between BMI and TNFα in subjects with BMI ≥40 differed significantly ($P < 0.05$) compared to that between subjects with BMI <40. The level of TNFα in GCF was positively correlated ($P < 0.05$) with BMI in subjects with no periodontal pathological pocket. No significant correlation was found between the level of IL-8 and BMI. The results indicate that BMI positively correlates with TNFα in GCF in the group of young subjects with BMI ≥40 as well as in the subjects with no pathological periodontal pocket (≥4 mm) and that TNFα in GCF may be affected by the obese condition through a systemic effect. □ *Crevicular fluid; IL-8; obesity; TNFα*

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The prevalence of obese children and adults is increasing dramatically worldwide (1, 2). About 30% of Swedish adolescents are overweight or obese and approximately 80% of the children who are obese remain obese throughout adulthood (3). The higher body mass index (BMI), expressed as body weight divided by height squared (4), the greater the risk of developing health problems, including type 2 diabetes, cardiovascular diseases, and certain cancer categories (5–8). Recent data on obesity and oral health indicate that obesity is also a risk factor for periodontitis, and a higher prevalence of periodontitis is found among obese patients (9–11). In addition, obesity, especially among younger individuals, might be a potential risk factor for periodontal disease (11), but the mechanism(s) behind this is unclear.

Adipose tissue, a dynamic endocrine organ, secretes a number of factors collectively known as adipokines, some of which contribute to the pro-inflammatory milieu, including systemic and vascular inflammation (12, 13). These include tumor necrosis factor α (TNFα), interleukin-6 (IL-6), interleukin-8 (IL-8), leptin, plasminogen activator inhibitor-1, resistin, and angiotensinogen (13–15). Many of these adipokines in plasma are enhanced during obese conditions (13, 14).

Among many inflammatory and immune mediators identified in gingival crevicular fluid (GCF), it has been suggested that cytokine TNFα plays an important part in the pathogenesis of periodontitis and an enhanced level of TNFα is reported in GCF in patients with periodontitis (16). In addition, the cytokine, IL-8, a potent neutrophil chemoattractant, produced in response to different pro-inflammatory stimuli including TNFα, is also enhanced in GCF in subjects with periodontal disease (17, 18). Since obesity contributes to a pro-inflammatory milieu by producing pro-inflammatory cytokines, we hypothesized that the levels of TNFα and IL-8 in GCF might be positively correlated to the degree of obesity in terms of BMI.

Materials and methods

The present study was conducted on 33 subjects (11 M and 22 F) referred to the Department of Pediatric Dentistry from the National Childhood Obesity Center, Huddinge University Hospital. All had a BMI within the obesity for age range according to Cole et al. (19). The subjects ranged in age from 13 to 24 years. Neither cardiovascular disease nor diabetes was diagnosed in any of them. One subject was diagnosed with Laurence-Moon-Bardet-Biedls Syndrome (an autosomal-recessive disorder characterized by obesity, polydactyly, retinal dystrophy, mental retardation, and hypogonadism), one had a mutation in the melanocortin 4 receptor gene (MC4R),

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and one had hypopituitarism that was controlled and substituted.

Body weight (kg) and height (cm) of the subjects were determined, and BMI (kg/m^2) was calculated. The subjects answered a questionnaire concerning smoking habits (number of cigarettes smoked per day, years smoked), and none had been on antibiotic treatment in the 3 months prior to examination. The Ethics Committee at Huddinge University Hospital, Karolinska Institutet approved the study protocol. All subjects gave written and/or oral consent before participating in the study.

Clinical examination

Gingival condition was described using the Gingival Bleeding Index (GBI %) (20) at the mesiobuccal, buccal, distobuccal, mesiolingual, lingual, and distolingual sites of all teeth present (wisdom teeth were excluded from the study). The index, GBI %, was calculated as a percentage of bleeding gingival sites in relation to the number of sites examined. Pocket depth (mm) was measured from the peak of the gingival margin at the mesiobuccal, buccal, distobuccal, mesiolingual, lingual, and distolingual sites of all teeth present using a graded periodontal probe (type LM Instruments). The pathological periodontal pocket was classified when the depth was $\geq$4 mm.

In order to detect alveolar bone loss, two to four bitewing or two periapical radiographs (regio 13–23 and regio 33–43) were taken using a standardized technique, long cone paralleling technique. The distance from the cemento-enamel junction (CEJ) to the alveolar bone crest (AC) on the mesial and distal surfaces of all teeth was measured with the assistance of a Peak scale loupe (Carton Optic, Tokyo, Japan; 7-fold magnification), which permits measurements to the nearest 0.1 mm. Alveolar bone loss was classified when the distance from CEJ to AC exceeded 2 mm (21).

Crevicular fluid samples

Gingival crevicular fluid (GCF μL) was collected at six sites (16, 11, 26, 36, 31, and 46) from each subject using a paper strip (Periopaper, Proflow Inc., Amityville, N.Y., USA). The strip was inserted into the gingival crevice and left for 30 s. The strip was then analyzed using Peritron 8000 sensors and the volume was calculated by interpolation from the standard curve and expressed as μL GCF. The periopaper was placed in 120 μL assay buffer containing 0.9% NaCl, 0.01 M EDTA, 0.3% bovine-globulin, 0.005% Triton-X-100, 0.05% sodium azide, 0.0255 M NaH_2PO_4, 0.0245 M Na_2HPO_4, pH 6.8 and kept frozen at −70°C.

The GCF samples were analyzed at the Research Department of Odontology, Huddinge University Hospital. Cytokine levels (pg/mL) were determined using commercially available ELISA kits and in accordance with the manufacturer's instructions. TNFα was purchased from Biosource International, Inc., USA and IL-8 from Quantikine R&D systems, Minneapolis, USA.

Table 1. Clinical data of the subjects (n = 32)

Variables	Mean ± s
Age (years)	17.8 ± 1.9
Gender (male/female)	11/21
BMI (kg/m^2)	38.6 ± 7.7
Smokers/non-smokers	9/23
GBI (%)	29 ± 26
Pathological periodontal pockets ($\geq$4 mm)	15/32
Alveolar bone loss	0/32

s = standard deviation.

Statistics

We employed statistical analysis using the Pearson correlation coefficient between BMI and different variables. When comparing the correlation coefficients, we used Z-transformation (22). Student's t test, unpaired, was used to compare the mean between the two groups.

Results

The clinical data of the subjects (n = 32) are given in Table 1. The mean number of pathological periodontal pockets ($\geq$4 mm) was 4.5 and the mean GBI was 29% within the whole group. No alveolar bone loss was detected among the subjects. Of the subjects examined, 15 exhibited one or more pathological pockets ($\geq$4 mm), whereas in the majority of cases (n = 17) no pathological pockets were detected. The mean value of BMI within the group was 38.6, and 12 of the subjects exhibited a BMI value >40. In a univariate model there was no statistical correlation between BMI and the variables age, number of periodontal pockets ($\geq$4 mm), smoking, TNFα, and IL-8 (Table 2).

The subjects were divided into two subgroups with respect to BMI >40 (45.2 ± 6.6) and <40 (34.6 ± 3.9). There were no differences regarding age, gender, smoking, pathological periodontal pockets and levels of the biochemical variables TNFα and IL-8 between the two groups (Table 3). Regarding the correlation between the cytokines and BMI, there was a positive significant

Table 2. Correlation coefficients between BMI and the variables studied in obese subjects (n = 32)

Variables	BMI (kg/m^2)	Significance
Age (years)	r = 0.40	NS
TNFα (pg/mL)	r = 0.02	NS
IL-8 (pg/mL)	r = 0.15	NS
GBI %	r = 0.24	NS
Pathological pockets ($\geq$4 mm)	r = 0.19	NS
Smokers	r = 0.12	NS

Table 3. Periodontal and biochemical variables in subjects with BMI <40 or BMI ≥40

	BMI <40 n = 20		BMI ≥40 n = 12		
Variables	Mean	*s*	Mean	*s*	Significance
Biochemical:					
GCF (μL)	0.4	0.1	0.4	0.1	NS
IL-8 (pg/mL)	131	107	151	88	NS
TNFα	3.6	2.7	2.4	2.4	NS
Periodontal condition:					
Pathological periodontal pockets	4.8	7.1	3.9	6.9	NS
GBI (%)	31	27	25	23	NS
Clinical:					
Age	17.2	2.1	19.4	2.9	NS
Smokers/nonsmokers	6/20		4/12		NS

s = standard deviation.

correlation ($r = 0.74$, $P < 0.01$) between TNFα in GCF and BMI in the subjects with BMI ≥40 (Fig. 1A). On the contrary, in subjects with BMI <40 there was no significant correlation between BMI and TNFα ($r = 0.18$, NS) (Fig. 1B). Moreover, when comparing the correlation coefficients between BMI and TNFα in the two subgroups, there was a statistical difference ($P < 0.05$).

The subjects were also divided into those with and those without any pathological periodontal pockets (≥4 mm) (Table 4). There was no statistical difference regarding age, GBI %, smoking habits, BMI, or the levels of TNFα and IL-8 in the two subgroups (Table 4). However, a significant positive correlation ($r = 0.51$, $P < 0.05$) was found between BMI and the level of TNFα in the subjects without any pathological periodontal pocket (Fig 2). No significant correlation was found between BMI and TNFα for subjects with pathological periodontal pockets ($r = -0.16$, NS).

Discussion

The aim of this study was to investigate the relationship between BMI and the levels of cytokines TNFα and IL-8 in GCF. This novel finding demonstrates a positive correlation between BMI and the level of TNFα in GCF from subjects with BMI >40 as well as from subjects with no pathological periodontal pockets (≥4 mm).

The subjects in this study exhibited good periodontal condition, since no early sign of alveolar bone loss was detected on the radiographs. It is not possible to evaluate the effect of BMI on periodontal condition since no control group was included.

The focus of this study was in investigating the levels of TNFα and IL-8 in GCF in relation to BMI. TNFα has been reported to be closely associated with obesity and the cytokine is produced not just by macrophages but also by the adipose tissue. The cytokine TNFα secreted by adipose tissue is assumed to be critical in insulin resistance and in the pathogenesis of non-insulin-dependent diabetes mellitus (23). So far, no study has been made available in the literature in which cytokines in GCF from obese subjects have been studied.

In a univariate model, there was no statistical correlation between BMI and the variables age, number of periodontal pockets (≥4 mm), smoking, TNFα, and IL-8. When separating the subjects with respect to BMI index, the level of TNFα in GCF was positively correlated with BMI in subjects with BMI >40. The level of TNFα in GCF did not differ between subjects with BMI higher or lower than 40. Altogether, the result suggests that the systemic effect in terms of obesity probably needs to be fairly high (BMI >40) before a relationship between TNFα in GCF and BMI is demonstrated. Interpretation of the results has to be made carefully, however, since the number of subjects is limited.

Based on our findings, we hypothesize that the relationship between TNFα and BMI among the most obese subjects may be due to a systemic effect of circulating TNFα from plasma. The assumption that TNFα in GCF derives from the adipose tissue through a systemic influence is compatible with the finding that obesity is a low-grade systemic inflammatory disease, but also that the periodontal condition in terms of GBI % did not differ between the two subgroups. However, we cannot rule out the possibility that the level of TNFα in GCF is to some extent affected by a local production of TNFα in the periodontal tissue. It is not possible to evaluate whether the GCF level of TNFα correlates to the serum level due to lack of serum samples in this study.

Regarding the association between IL-8 and BMI, we did not demonstrate any significant correlation between IL-8 in GCF and BMI in a univariate analysis either within the whole group or in the subgroups. This is compatible with the view that the adipose tissue has a minor impact on the circulating levels of IL-8 (24). The fact that IL-8 was not correlated with BMI further supports the concept of a systemic influence of obesity on the level of TNFα in GCF.

When the subjects were divided into the two subgroups with respect to the occurrence of pathological periodontal pockets (≥4 mm), the GCF level of TNFα was significantly positively correlated with BMI in subjects with no pathological pocket. This finding further supports our assumption that the relationship between TNFα and BMI observed in the most obese subjects is mainly due to a systemic effect rather than that TNFα in GCF is derived from monocytic cells related to inflammatory periodontal condition. It is possible that the cytokine TNFα negatively affects the local immunity in the periodontal tissue, which might be a possible link whereby obesity acts as a risk factor for periodontal disease, recently reported in human studies (9–11). Moreover, TNFα produced as a result of periodontal inflammation might also be an additional important factor influencing insulin sensitivity in obese subjects (25). Whether TNFα production from adipose

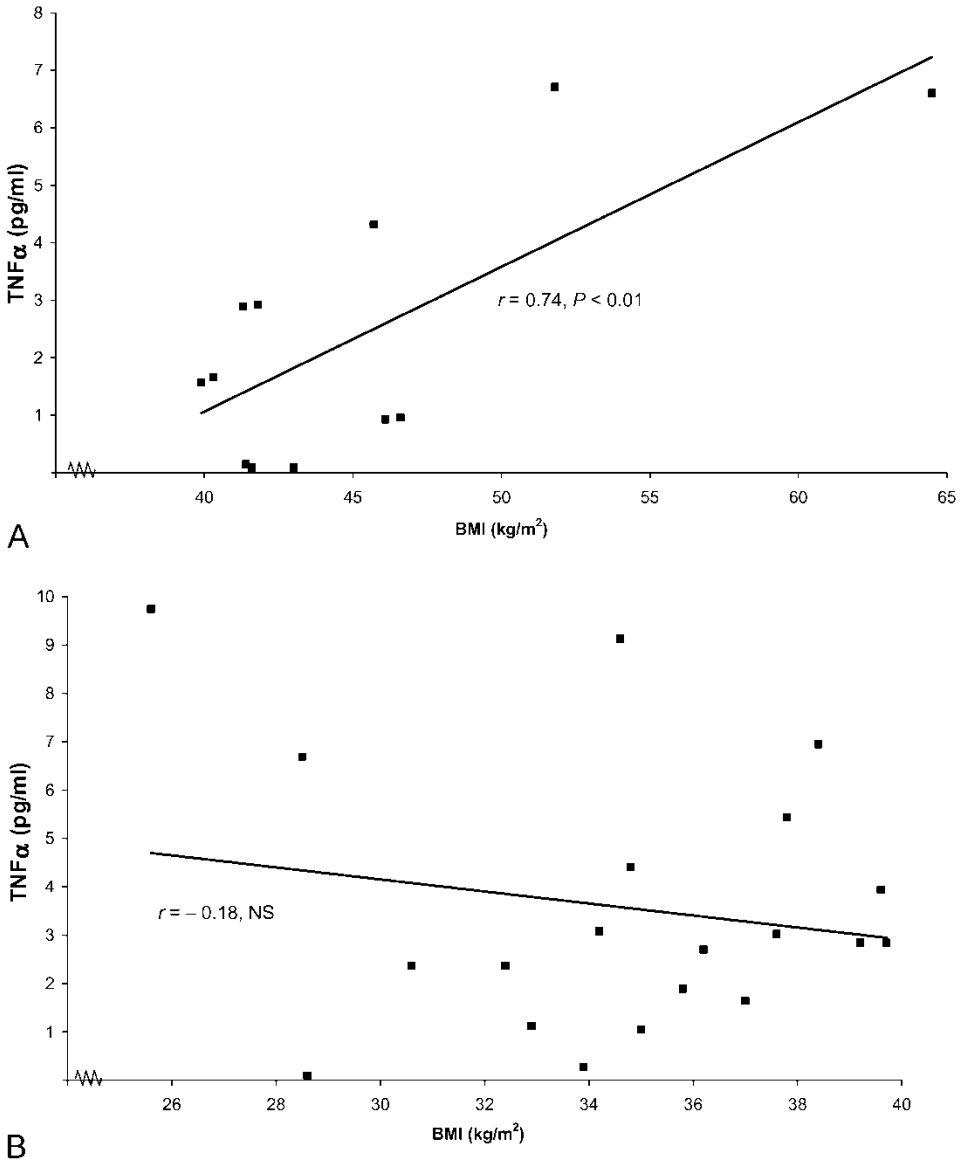

Fig. 1. Scatter diagram illustrating the correlation between BMI (kg/m^2) and TNFα (pg/mL) in GCF (μL) in subjects (A) with BMI ≥40 (n = 12) and in subjects (B) with BMI <40 (n = 20).

Table 4. Clinical and biochemical variables in subjects with or without pathological periodontal pockets (≥4 mm)

	With pathological pockets (n = 15)		Without pathological pockets (n = 17)		Significance
Variables	Mean	s	Mean	s	NS
Age	18.3	2.6	17.1	2.3	NS
GBI %	44	28	16	14	NS
BMI (kg/m^2)	38.5	5.2	38.9	9.5	NS
Smokers/non-smokers	3/15		6/17		NS
GCF (μL)	0.4	0.1	0.4	0.1	NS
IL-8 (pg/mL)	152	76	124	113	NS
TNFα (pg/mL)	2.6	1.8	3.9	3.2	NS

s = standard deviation.

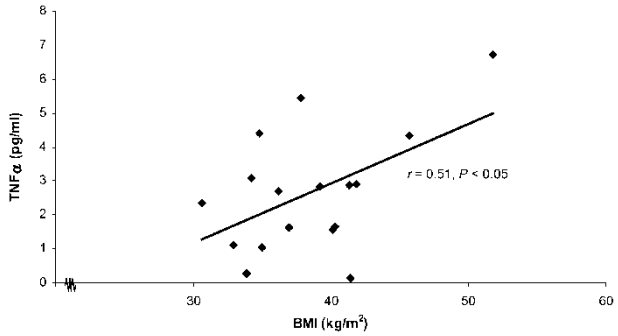

Fig. 2. Scatter diagram illustrating the correlation between BMI (kg/m^2) and TNFα (pg/mL) in GCF (μL) in subjects with no pathological periodontal pocket.

tissue contributes to an enhanced risk for periodontal inflammation is an interesting hypothesis which has to be evaluated in longitudinal studies.

In conclusion, the results indicate that BMI positively correlates with TNFα in GCF in the group of young subjects with BMI >40 as well as in the subjects with no pathological periodontal pockets. Furthermore, the level of TNFα in GCF may be affected by the obese condition through a systemic effect.

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