The Correlation of Serum and Gingival Crevicular Fluid Cytokines in Obese Subjects

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Abstract

Objective: To investigate the correlation between the gingival crevicular fluid (GCF) levels of IL-6 and TNF-α with the levels in serum in obese patients. **Design:** Twenty-six obese adults (BMI ⩾ 30, age 33-74) provided serum and GCF samples. Smokers and uncontrolled diabetics (HbA1c > 8%) were excluded. Serum and GCF samples were analysed for IL-6 and TNF-α using commercially available ELISA kits. Within each subject GCF was collected from two healthy sites (n = 26 subjects) and two gingivitis sites, defined by bleeding on probing (n = 22 subjects). The levels of IL-6 and TNF-α in the GCF were compared and correlated with the levels found in serum using Spearman's correlation analysis. A Bland-Altman analysis was used to determine the level of agreement between serum and GCF samples. **Results**: IL-6 was more frequently detected than TNF-α. This was consistent in serum (100% vs 64%) and GCF samples from healthy (73% vs 52%) and gingivitis (95% vs 36%) sites. There were no significant correlations between the TNF-α in serum and GCF samples from healthy ($r = 0.27$, $p = 0.22$) and gingivitis ($r = -0.19$, $p = 0.40$) sites. In contrast, positive correlations were found for IL-6 between serum and GCF samples from healthy ($r = 0.48$, $p = 0.03$) and gingivitis ($r = 0.79$, $p = 0.0001$) sites. The correlation and agreement was strongest for IL-6 between serum and gingivitis GCF samples. **Conclusion**: The results of this pilot study suggest a lack of correlation and poor agreement between serum and GCF samples in obese subjects. Studies examining the link between periodontitis and obesity should consider collecting both serum and GCF.

Key words: Obesity, periodontal disease, gingival crevicular fluid, serum, cytokines

Introduction

Obesity is a rapidly emerging health concern and now recognised as a low-grade inflammatory disease. It has recently been associated with periodontitis (Al-Zahrani *et al*., 2003; Dalla Vecchia *et al*., 2005; Saito *et al*., 1998; Saxlin *et al*., 2010; 2011; Ylöstalo *et al*., 2008) and may confound the link between periodontitis and cardiovascular disease. These diseases share common risk factors but may be linked by a pro-inflammatory state (Nishimura and Murayama 2001; Pischon *et al*., 2007; Saito and Shimazaki 2007). The pro-inflammatory cytokines released from adipose tissue may influence the gingival inflammatory response to plaque. However, before this can be established, correlations between the levels of such cytokines in serum and gingival crevicular fluid (GCF) should be examined.

Recently, the TNF-α levels in GCF have been investigated in obese children (Khosravi *et al*., 2009) and adolescents (Lundin *et al*., 2004). The TNF-α levels in GCF samples were positively correlated with the body mass index (BMI). In both studies, the serum levels of TNF-α were not evaluated, and therefore it is not clear whether GCF TNF-α levels truly reflect the levels found in serum.

IL-6 and TNF-α cytokines are implicated in the destructive process of periodontitis (Graves 2008) and are released locally and systemically from macrophages present within adipose tissue (Hotamisligil *et al*., 1993; Weisberg *et al*., 2003). Consequently, the serum levels of TNF-α and IL-6 are increased in obese subjects and a positive correlation exists between the serum level of IL-6 and the subject's BMI. In contrast, TNF-α is

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elevated but does not show a correlation with the BMI. This difference was explained by the presence of soluble TNF-α receptors (Mohamed-Ali *et al.*, 1999) which may bind or deactivate the TNF-α prior to reaching the GCF, and therefore limit its biological impact.

The correlations between serum and GCF levels of IL-6 and TNF-α have not been investigated. Previous investigations have demonstrated that IL-6 and TNF-α are not always detected in the serum of non-obese subjects (Gorska *et al.*, 2003; Loos 2005). Obese subjects are an ideal population in which to evaluate these correlations because they have an abundant source of systemic cytokines, meaning that a lack of detection might be less common.

Therefore, the aim of this pilot study was to investigate the correlation between GCF and serum levels of IL-6 and TNF-α in obese subjects. This will provide preliminary data to help determine whether a relationship exists between the adipokines found in serum and the amount released into the GCF, thus partially supporting a biological connection between obesity and periodontitis.

Materials and methods

Experimental design

Recruited subjects underwent a periodontal examination, which included collection of GCF and serum. ELISA was used to examine the levels of IL-6 and TNF-α in GCF and serum samples to investigate a possible correlation between the serum and GCF cytokine levels.

A descriptive cross-sectional examination was used to analyse the levels of IL-6 and TNF-α in serum and GCF samples. The cytokine levels were compared within each subject to determine the presence of a correlation between GCF and serum samples. Ethical approval was obtained from the Sydney West Area Health Service Human Research Ethics Committee [Ref HREC2009/2/4.6(2920)].

Study population

Subjects were recruited from an obesity clinic at Westmead Hospital, Australia. The clinic provides a multi-disciplinary approach to the treatment of obesity. In addition to monitoring the weight of obese patients, the clinicians diagnose and manage obesity-related conditions, such as diabetes and cardiovascular disease. Patients attending dental clinics in the Westmead Centre for Oral Health with a BMI $\geqslant$ 30 were also invited to participate in the study. Verbal and written explanations were given to all participants and consent was obtained. Detailed inclusion and exclusion criteria are presented in *Table 1*.

Clinical measures

Recruited patients attended the Periodontics clinic at Westmead Centre for Oral Health. Their periodontal status was visually examined to assess possible GCF collection sites. Gingival crevicular fluid sample collection was followed by a comprehensive periodontal examination including recording of clinical attachment level (CAL), probing pocket depth (PPD) and bleeding on probing (BOP) at six sites per tooth. Third molars were not included. Gingival crevicular fluid samples were collected prior to periodontal charting to avoid stimulation of the gingival tissues and blood contamination. Immediately following the successful collection of GCF and periodontal charting, the subjects were sent for venous blood sample collection within 60 minutes.

GCF sample collection

Gingival crevicular fluid samples were required from two healthy (non-bleeding) sites for each subject. A variable number of additional samples were taken from different sites to compensate for samples that were later excluded because of BOP. Another two samples were taken from sites showing clinical signs of gingival inflammation (subsequently assessed by BOP and PPD $\leqslant$ 4 mm). Sampling sites were isolated with cotton rolls. Supragingival plaque was removed with a periodontal probe and cotton pellets. The area was gently air-dried and filter paper strips were placed in approximal locations for 30s. A single standardised periopaper strip (Proflow, Oraflow Inc. Plainview, NY, USA) was inserted into the gingival crevice but did not exceed 1 mm deep. Blood contaminated strips were discarded and new sites were selected. Each sample was placed into separate microcentrifuge tubes for storage at -20°C. The samples were transferred to -80°C within four hours, where they were stored until further analysis (Khosravi *et al.*, 2009; Tüter *et al.*, 2007).

Serum sample collection

Immediately following the collection of GCF and periodontal charting the subjects attended the blood collection service of the Institute of Clinical Pathology and Medical Research (ICPMR), Westmead Hospital, Australia. Ten ml of venous blood was obtained within 60 minutes from the antecubital fossa using serum tubes (BD Vacutainer® Serum Tubes, North Ryde, NSW, Australia). These tubes were transferred to the Institute for Dental Research, Westmead Centre for Oral Health, where they were left at room temperature for 30 minutes to maximise clot contraction. In the case of incomplete clot contraction, the clot was separated from the glass tube using a sterile cotton swab and incubated for a further 30 minutes at 37°C (Sanyo CO_2 incubator, Sanyo, North Ryde, NSW, Australia).

The samples were subsequently chilled on ice for 90 minutes prior to centrifuging at 1400 rpm (Eppendorf 5810R, Eppendorf South Pacific, North Ryde, NSW, Australia) for 5 minutes at 4°C. The

superficial supernatant was removed using a 1 mL micropipette and stored in microcentrifuge tubes at -80°C (Thermo Ultra-low temperature freezers, Thermo Scientific, K.I. Scientific, Lane Cove, NSW, Australia) until the day of ELISA analysis. Separate serum tubes were sent to the ICPMR for analysis of HbA1c (in diabetics) and fasting blood glucose (in non-diabetics).

ELISA analysis - GCF

Cytokines were eluted from the thawed filter paper with 100 μL of phosphate buffered saline (PBS). A vortex mixer (SVM1, Selby Biolab, Clayton, Victoria, Australia) was used to assist elution of cytokines from each filter paper. Two 10-second intervals were used. A micro-centrifuge (Heraeus Biofuge 15, K.I. Scientific, Lane Cove, NSW, Australia) was used for 5 minutes at 2000 rpm to further enhance mixing. Each GCF sample was subsequently analysed for IL-6 and TNF-α, in duplicate, using commercially available ELISA kits (Quantikine, R&D Systems, Minneapolis, MN, USA).

Briefly, 20 μL of each sample (or standard) was added to microplate wells. Phosphate buffered saline was added to make up a volume of 100 μL for IL-6 and 200 μL for TNF-α. Reagents were added sequentially according to the manufacturer's instructions. Because of the formation of bubbles in the reagents and loss of volume, 80% of each reagent was added to the ELISA plates. This was consistent in all sample processing. An orbital shaker (Bioline Shaker, Adelab, Thebarton, SA, Australia) was used during incubation in accordance with the ELISA kit instructions; however, the speed was reduced to 120 rpm.

After the recommended incubation times, the absorbance was measured at 490 nm using a microplate reader (Bio-Rad Benchmark Microplate Reader, Bio-Rad Laboratories, Regents Park, NSW, Australia). Absorbance correction was made at a wavelength of 655 nm. The minimum detectable doses according to the manufacturer were 0.039 pg/mL for IL-6 and 0.106 pg/mL for TNF-α. Results were recorded as a total amount of cytokines (in pg) per 30s sample for each subject.

ELISA analysis - serum

Serum samples were thawed and centrifuged at 2000 rpm for 5 minutes along with the GCF samples. Thereafter, 20 μL of standard or serum were added to the ELISA kit containing the corresponding GCF sample for each subject to avoid variation between assays. The reagents were added exactly as described above. Results were recorded as serum concentrations of cytokines (in pg/mL) for each subject.

Statistical analysis

The distribution of cytokine levels in serum and GCF were examined to identify potential outliers. Because of the small sample size, we could not assume normal distribution of our data and, therefore, the relationship between cytokines (IL-6, TNF-α) in serum and GCF were estimated using Spearman's correlation analysis. Statistical significance of this coefficient was determined using two-sided p values ($p < 0.05$). Furthermore, agreement of the cytokine values between GCF and serum were evaluated using a Bland-Altman analysis. This test quantifies inter-method agreement for individual patients over the entire range of clinical values of a given parameter (Bland and Altman, 1999). In the present study, this method was used to measure agreement between serum and GCF cytokine levels within each subject.

Initially, ten samples were used to confirm laboratory procedures. At this stage, it was noted that there were a high number of GCF samples below the detection threshold. The inability to detect a cytokine at a gingival site despite its presence in serum supports a low correlation. Therefore, samples below the detection threshold were given arbitrary values of detection limit/$\sqrt{2}$.

Results

Subject recruitment

A total of 46 subjects agreed to undergo a dental examination and 39 of them initially met the inclusion criteria. One subject was considered a poorly controlled diabetic (HbA1c > 8%) and the data were excluded. Another subject could not wait for serum collection and, therefore, the GCF data were excluded. One serum sample had incomplete data on diabetic status and the subject was excluded. No adverse events were recorded for GCF or serum sample collection. The samples from the remaining 36 subjects were processed using the ELISA technique. The first 10 samples were excluded due to changes in laboratory protocol as a result of the preliminary study findings, which altered the dilution procedures. Therefore, the data from 26 subjects were included in the final analysis.

Subject demographics

Subject variables are summarised in *Table 2*. Non-diabetic subjects had a fasting blood sugar level (BSL) ⩽ 6 mmol. Subject 7 had a mildly elevated BSL (7 mmol) which is an indication of insulin resistance and is the cut-off value for a diagnosis of type 2 diabetes (American Diabetes Association, 2010). This subject, however, had an HbA1c level of 6.7%, which was within the inclusion criteria of the current study.

Clinical findings

Twenty-one of the 26 subjects had > 90% of sites with shallow probing depths (PPD ⩽ 3mm). This reflects the low prevalence of periodontitis in the study subjects according to the criteria set out by Tonetti and Claffey (2005). Four subjects had healthy gingival

Table 1. Inclusion and exclusion criteria

Inclusion criteria

- ⩾ 18years of age and the ability to give informed consent
- BMI ⩾ 30 kg/m^2
- Minimum of two healthy gingival sites for GCF sampling
- Diagnosed with periodontitis, gingivitis or periodontal health

Exclusion criteria

- Current smokers (⩾ 1 cigarette/day)
- Poorly controlled diabetes (HbA1c ⩾ 8%)
- Patients requiring antibiotic prophylaxis prior to dental treatment
- Antibiotics or systemic corticosteroids within the last month
- Periodontal therapy within the last six months
- General scaling in the last month
- Current inflammatory or infectious disease
- Current medications influencing the periodontal tissues
- Current pregnancy or breast feeding

BMI, body mass index; GCF, gingival crevicular fluid

Table 2. Summary of subject variables

Subject Variables	Mean ± SD (Range)
Mean age ± SD (range)	53 ± 11.13 (33-74)
Sex (females)	17/26
Diabetes (type 2)	10/26
Mean fasting BSL ± SD (mmol/L)	4.98 ± 0.70 (4.1-7.0)
Mean HbA1c ± SD (%)	6.42 ± 0.80 (5.5-7.9)

BSL, blood sugar levels; SD, standard deviation

conditions in all accessible sites, and therefore,\ no gingivitis samples were taken (subjects 7, 12, 13 and 26). The mean BOP value was 33% (range 7-74%). Periodontal variables and relevant medical conditions are shown in *Table 3*.

Cytokine detection

The proportion of samples above the ELISA detection limit was greater in serum when compared with GCF samples. *Table 4* summarises the detection frequency of cytokines in serum and the GCF from healthy and gingivitis sites.

The median GCF cytokine levels are presented in *Table 4*. No significant differences were found between the median amounts of IL-6 collected from healthy or gingivitis GCF samples ($p = 0.15$). Similarly, the median amount of TNF-α in GCF samples did not differ between sites ($p = 0.22$). The level of TNF-α in subject 19 was not consistent with the other samples and was considered an outlier in subsequent analyses.

Correlation analysis

The serum concentration of IL-6 was significantly correlated to the amounts found in the GCF samples. The IL-6 correlation was highest for gingivitis samples ($r = 0.79$, $p = 0.0001$) compared with the GCF from healthy sites ($r = 0.48$, $p = 0.03$). There was no correlation for TNF-α in gingivitis ($r = -0.19$, $p = 0.40$) and healthy ($r = 0.27$, $p = 0.22$) samples. A subgroup analysis based on gender revealed significant correlations between serum and IL-6 in healthy GCF samples in males ($r = 0.83$, $p = 0.02$) and between serum and IL-6 from gingivitis GCF samples ($r = 0.87$, $p < 0.0001$) in females. There were no other significant correlations, possibly because of the small number of subjects in each gender subgroup. The main correlation graphs are shown in *Figure 1*. The relationship between serum and GCF samples varied between subjects and this is illustrated in the Bland-Altman analysis.

Bland-Altman analysis

The Bland-Altman analysis is presented in *Figure 2*. The mean and 95% limits of agreement are represented by the horizontal lines. The graphs illustrate, on a log scale, that there was generally poor agreement and high variability between cytokine levels in serum and GCF

Table 3. Periodontal variables and relevant medical conditions and medications of each subject

Subject	Sex	Medical Conditions/Medication					Smoking	Percentage of		
		OA	COX	OSA	AB	OTHER		BOP	PPD 1-3 mm	PPD 4-6 mm
7	M						Never	17%	97%	3%
12	F						Never	26%	97%	3%
13	F	Y					Never	52%	73%	26%
14	F	Y					Never	63%	90%	10%
15	F	Y				Actonel	Never	58%	52%	47%
16	F						Never	39%	100%	0%
17	F	Y	Y	Y			Never	44%	100%	0%
18	M		Y	Y	Y		Former	9%	100%	0%
19	M	Y					Never	74%	63%	35%
20	F	Y	Y			Fosamax	Former	32%	91%	8%
21	F	Y		Y			Former	22%	87%	13%
22	F						Never	7%	100%	0%
23	F	Y					Former	17%	100%	0%
24	M						Never	39%	96%	4%
25	F	Y			Y	Prednisone 6weeks prior	Never	23%	100%	0%
26	M			Y			Former	11%	100%	0%
27	M						Never	35%	95%	5%
28	F	Y				Plavix	Never	29%	100%	0%
29	M			Y			Never	41%	75%	25%
30	F	Y		Y			Former	48%	91%	9%
31	F				Y		Former	14%	99%	1%
32	M			Y			Former	17%	100%	0%
33	F			Y		Methotrexate	Never	38%	98%	2%
34	F	Y		Y			Never	32%	99%	1%
35	M	Y		Y			Never	22%	98%	2%
36	F						Former	44%	97%	3%

OA, osteoarthritis; COX, cyclooxygenase inhibitors (e.g. aspirin, NSAIDs); OSA, obstructive sleep apnoea; AB, antibiotics within 3 months

samples. Log-transformed IL-6 levels in serum and gingivitis samples showed a more consistent distribution around the mean; however, the wide 95% limits indicated weak agreement between the two parameters.

Discussion

To the best of our knowledge, no previous studies have evaluated the correlations between IL-6 and TNF-α in the GCF and serum of obese subjects. Given the absence of prior data that were directly relevant to the present study, a formal power calculation was not possible, and therefore the study was conducted as a pilot study.

The aim of our study was not to compare the mean GCF cytokine levels from periodontitis, gingivitis or healthy sites in obese subjects but to investigate the correlation and agreement between serum and GCF cytokines in obese subjects. If there is no correlation between the serum and GCF cytokines, further studies investigating GCF cytokine levels in obese subjects may need to take the serum cytokines levels into consideration. Results of the current study showed a statistically significant correlation between serum and GCF samples was found for IL-6 but not TNF-α. Despite the positive correlation, agreement was poor. This might suggest that the relationship is influenced by an unknown factor or inter-individual variability.

A second finding in this study was the higher correlation and agreement between serum and GCF samples taken from gingivitis sites than the corresponding samples from healthy gingival sites. This might infer that a higher level of systemic inflammation influences the inflammatory reaction in the gingiva. Alternatively, it could be argued that gingivitis has a systemic effect, as described previously (Andriankaja *et al.*, 2009). The current study is unable to establish the direction of this relationship and hence, further research may be needed.

It has been suggested that the cytokines released by adipose tissue might up-regulate the immune response to plaque bacteria (Pischon *et al.*, 2007). Pro-inflammatory cytokines are involved in inflammatory cell recruitment, and therefore a systemic source might

Figure 1. Correlation graphs for IL-6 and TNF-α (Spearman's coefficient shown on graph)

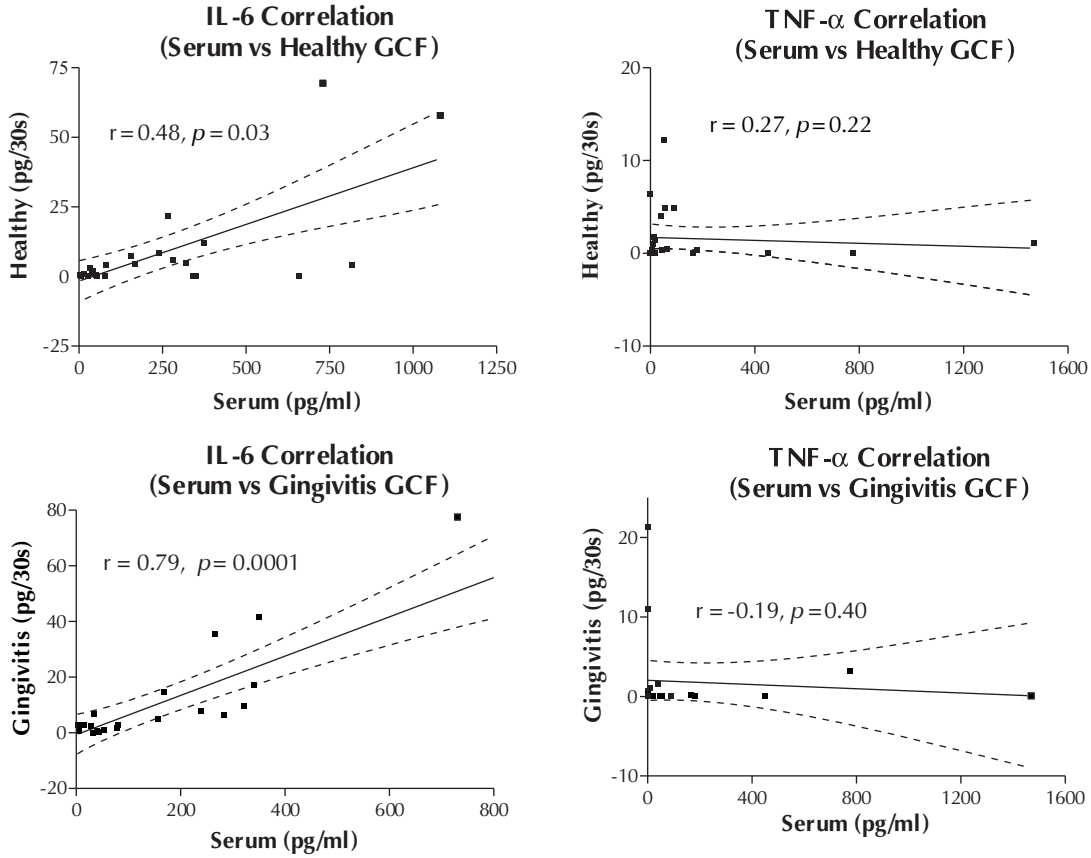


Table 4. Summary of cytokine detection rate and median values (pg/30 second sample)

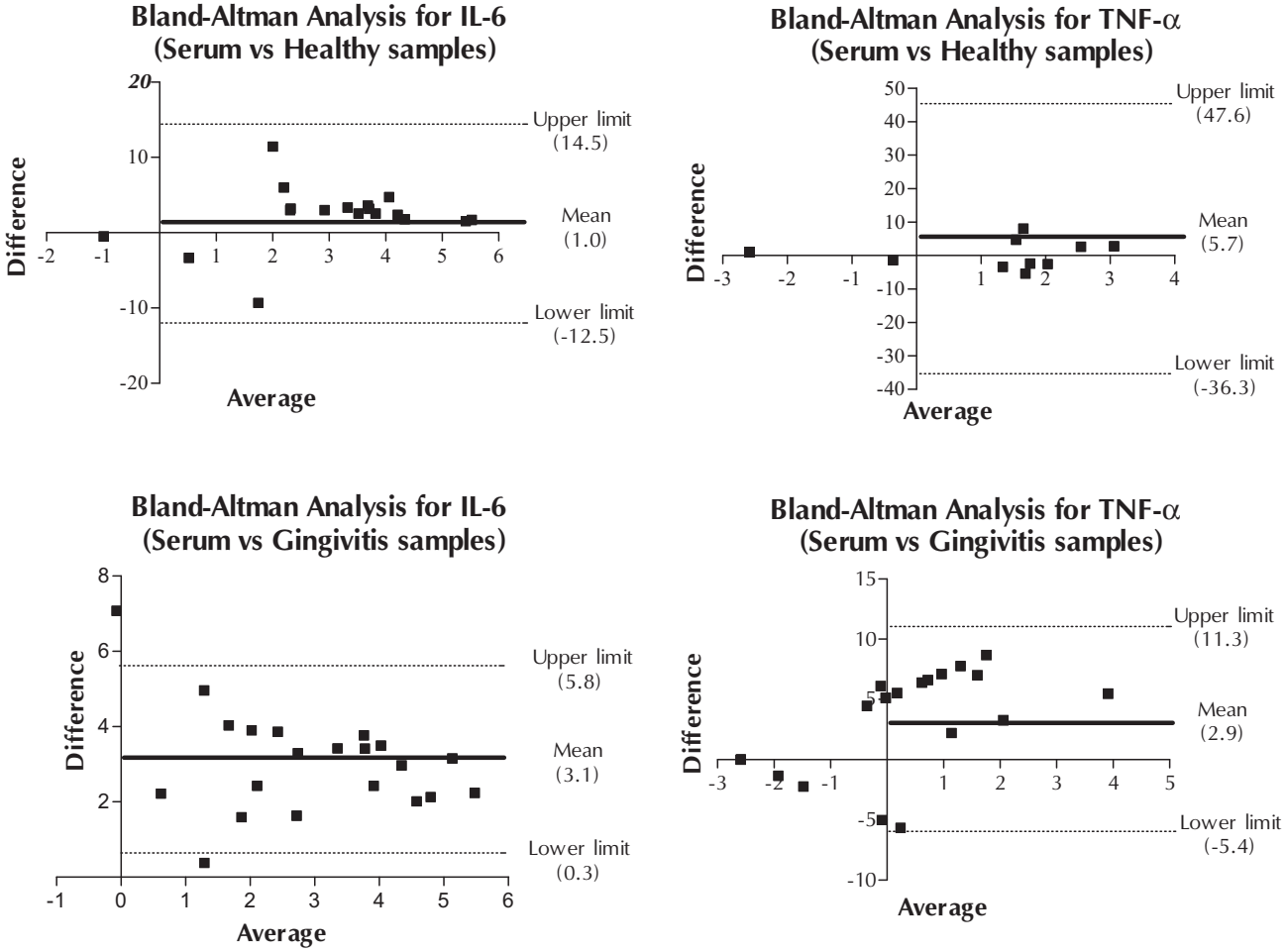
	IL-6		TNF-α	
	Detection rate	Median ± IQR*	Detection rate	Median ± IQR*
Serum	100%	157.593 ± 300.06 (n = 26)	64%	18.983 ± 62.56 (n = 2)
HealthyGCF sites	73%	3.084 ± 6.691[a] (n = 26)	52%	0.319 ± 1.366[b] (n = 25)
Gingivitis GCF sites	95%	5.818 ±14.925[a] (n = 22)	36%	0.075 ± 0.514[b] (n = 22)

*IQR = interquartile range; Mann-Whitney U test, $p = 0.15$[a], $p = 0.22$[b]

lead to a heightened inflammatory response in the gingiva. Animal studies have shown that exogenous administration of TNF-α (Gasperic *et al.*, 2003) and IL-1 (Koide *et al.*, 1995) leads to more pronounced inflammation and periodontal destruction. Gingival crevicular fluid represents a blood ultrafiltrate (Goodson, 2003). Hence, an increase in serum cytokines might lead to greater amounts in the gingival tissue and GCF. The presence of IL-6 mRNA confirms that cytokines are produced locally in healthy and diseased gingival tissue (Yamazaki *et al.*, 1994). It is not known whether this process is enhanced by increased serum pro-inflammatory cytokines. Based on the current investigation, it cannot be assumed that serum cytokines influence the gingival tissue because these mediators were not detected in the GCF of all subjects.

Previous reports on the serum levels of TNF-α and IL-6 in non-obese subjects were limited by the number of samples below the detection threshold (Boström *et al.*, 1999; Gorska *et al.*, 2003). As a consequence, little is known about the relationship between the serum and GCF levels of IL-6 and TNF-α. Loos (2005) suggested that IL-6 might only be detected in up to 53% and 42% of serum samples from periodontitis and healthy subjects, respectively. Similarly, Gorska *et al.*, (2003) showed TNF-α was only detected in 50% of serum

Figure 2. Bland-Altman analysis for IL-6 (left) and TNF-α (right)



samples. The low serum levels may result in a low rate of detection in GCF unless they are produced locally. In contrast, our study showed that even with high levels of IL-6 in serum, the levels were not always correspondingly high in GCF samples from healthy gingival sites. Two previous investigations (Khosravi *et al.*, 2009; Lundin *et al.*, 2004) assumed that serum cytokines reached the GCF. However, the results of the current study question this assumption.

Because of the difficulty in finding suitable control subjects it was not possible to evaluate differences in the prevalence of periodontitis or to compare the cytokine levels between obese and non-obese subjects. Obese adults are a difficult population to study because of the presence of multiple co-morbidities and various psychological traits as a consequence of excess body weight (Dumitrescu and Kawamura, 2010). The current patient group was chosen to investigate a principle, that is, the correlation of IL-6 and TNF-α between GCF and serum. This population was the ideal group to study, as the abundant cytokines in serum would reduce the chance of having cytokine levels below the detection threshold. The enrolled subjects had varying degrees of atherosclerosis, hypercholesterolaemia, osteoarthritis and obstructive sleep apnoea. All of these conditions are associated with increased serum pro-inflammatory cytokine levels (Hatipoglu and Rubinstein, 2003; Lind, 2003; Stannus *et al.*, 2010; Williams *et al.*, 1998) but there are no data regarding GCF. The current study minimised the impact of these co-morbidities by using the subject as his/her own control, where the serum and GCF cytokine levels were compared on an individual basis.

Diabetes mellitus was highly prevalent among the subjects recruited for the current study. The decision to exclude subjects with an HbA1c > 8% was based on a previous study that showed that the amounts of IL-1β in GCF were higher in poorly controlled diabetics (Engebretson *et al.*, 2004). The impact of periodontal disease status (periodontitis versus gingivitis) on the current results remains untested. The enrolled patients in this study were generally from a lower socioeconomic background who demonstrated poor dental awareness and health behaviour. Despite this, the presence of periodontitis according to the criteria recommended by Tonetti and Claffey (2005) was very low.

In the absence of data suggesting a superior GCF sampling technique, the filter paper method was chosen because the cytokines in this study have been

successfully recovered by various research groups (Boström *et al.*, 1999; Engebretson *et al.*, 2004; Giannopoulou *et al.*, 2003; Khosravi *et al.*, 2009; Lundin *et al.*, 2004). Gingival crevicular fluid sampling is technique-sensitive and can be influenced by the duration (Curtis *et al.*, 1988) and location of sample collection (Egelberg and Attström 1973) as well as the method of data presentation (Lamster *et al.*, 1988). The volume of gingival fluid was not quantified in this study to avoid errors associated with fluid evaporation or oversaturation of the filter strip. Most significantly, when measuring GCF volume it should be remembered that gingival inflammation increases fluid flow and, therefore, can dilute the sample (Daneshmand and Wade, 1976; Lamster *et al.*, 1986). For this reason the current protocol was developed to record the amount of cytokine collected within a 30-second period. While it may have inherent limitations, a previous report suggested that it provided the most consistent data (Lamster *et al.*, 1988).

As there have been no previous studies reporting the correlation between serum and GCF IL-6 and TNF-α cytokine levels, sample size calculation was difficult to conduct. Hence, the present study was designed to be a pilot study to provide some initial data for future investigations. Previous studies comparing antibody levels between serum and GCF had approximately 20 subjects (Smith *et al.*, 1985; Tew *et al.*, 1985), which was used to guide the required number of subjects in the present study. In the current study an arbitrary value (detection limit/2) was given for samples below the ELISA detection threshold rather than recording them as zero. The lack of detection does not imply the absence of the cytokine, rather, that it is below the detection limit of the ELISA assay. Eliminating these sites from statistical analysis would bias the results towards those having a good correlation.

The mean levels of cytokine evaluated in the current study should not be compared with those reported previously. The standard curve was constructed using known values provided by the manufacturer for each individual plate. Unfortunately, the standard curve did not obey the Beer-Lambert law (Commoner and Lipkin, 1949) for the range of cytokines detected in this study. Because of this, some readings were taken from the non-linear sections of the standard curve, and therefore may not be accurate. Serum and GCF samples for each subject were taken from the same ELISA plate, which may minimise this impact. However, future research is needed to confirm these pilot results.

The results of the present study suggested that, in obese subjects, the levels of TNF-α in serum were not correlated to the amounts in GCF from healthy and gingivitis sites. In comparison, IL-6 showed statistically significant correlations. The Bland-Altman analysis suggested poor agreement between serum and GCF samples for both TNF-α and IL-6. These results have implications for future research that aims to investigate the distant impact of systemic inflammation, especially when GCF and serum cytokines are evaluated independently. Future studies examining the effect of systemic cytokines on the periodontium should consider evaluating both serum and GCF. This would allow differentiation of the cytokines arising from the serum versus those produced locally. It will also help to determine if the cytokines present in the serum remain at sufficient levels to have an impact on the gingival tissue.

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