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Do patients with aggressive periodontitis have evidence of diabetes? A pilot study

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Background and Objective: Complex relationships exist between diabetes and periodontal disease. Diabetes is accepted as a risk factor for periodontal disease, and recent evidence supports the existence of a bidirectional relationship between these two diseases. It has been hypothesized that inflammation, lipids and adipokines may mediate these relationships. However, research regarding the above relationships with respect to aggressive periodontitis is very limited. This pilot study aimed to investigate whether patients with aggressive periodontitis (not previously diagnosed with diabetes) have evidence of diabetes and have altered serum levels of inflammatory mediators, lipids and adipokines.

Material and Methods: Glycaemic control markers (random plasma glucose and glycated haemoglobin), inflammatory mediators (high-sensitivity C-reactive protein, tumour necrosis factor-α, interleukin-1β, interleukin-6, interferon-γ and interleukin-18), lipids (triglycerides, total cholesterol and high-density lipoprotein-cholesterol) and adipokines (leptin, adiponectin and resistin) were measured in serum samples from 30 patients with aggressive periodontitis and 30 age- and sex-matched periodontally healthy control subjects, none of whom had a previous diagnosis of diabetes.

Results: Levels of glycaemic control markers, inflammatory mediators, lipids and adipokines were not significantly different ($p > 0.05$) between the aggressive periodontitis patients and healthy subjects for unadjusted and adjusted analyses (adjusting for body mass index, smoking, ethnicity, age and sex). The *p*-value for the adjusted analysis of adiponectin in female aggressive periodontitis patients compared with the female control subjects reached 0.064, the mean adiponectin level being lower in the female aggressive periodontitis patients (4.94 vs. 5.97 μg/mL).

Conclusion: This pilot study provided no evidence to suggest that patients with aggressive periodontitis (not previously diagnosed with diabetes) have evidence of diabetes or altered serum levels of inflammatory mediators, lipids and adipokines.

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Aggressive periodontitis is a specific form of periodontal disease. Clinically, it is distinguishable from other forms of periodontal disease primarily by its rapid rate of destruction and progression, its early age of onset or detection, its specific patterns of destruction and its familial aggregation. The aetiology and pathogenesis of aggressive periodontitis is not entirely clear, but there is evidence that specific microbiological, immunological and genetic features exist which are distinct to aggressive periodontitis (1).

Substantial research has linked diabetes with chronic periodontitis, but studies focusing on aggressive periodontitis are limited. It is widely

accepted that people with diabetes exhibit an increased prevalence and severity of periodontal disease and that the severity of periodontal disease correlates with levels of diabetes control (2). Furthermore, in nondiabetic people, increased fasting plasma glucose and glycated haemoglobin (HbA_{1c}) levels have been found to be significantly associated with periodontal disease (3,4). With regard to aggressive periodontitis, research is limited to a study by Nibali *et al.* (5) who found that a sample of nondiabetic patients with either aggressive periodontitis or severe chronic periodontitis exhibited higher nonfasting glucose levels than healthy control subjects. Anecdotally, some dentists consider testing for diabetes in patients presenting with aggressive periodontitis.

Conventionally, research has focused on a unidirectional relationship between diabetes and periodontal disease, in which diabetes predisposes to periodontal disease, and features observed in diabetes, such as defective polymorphonuclear neutrophils, altered vascular function and altered collagen metabolism, have been proposed to explain this relationship (6). However, in recent years, evidence has supported the existence of a bidirectional relationship between diabetes and periodontal disease (7,8), and it has been proposed that common susceptibility may exist between these two diseases (9,10). Hypotheses have developed regarding a role for inflammation, dyslipidaemia and adipokines in these relationships (5,10–17).

Both periodontal disease and diabetes have been associated with increased levels of systemic inflammatory mediators (18–21). Moreover, some evidence has suggested that diabetes may predispose to periodontitis by inducing a heightened inflammatory response (22), that low-grade systemic inflammation can result from periodontitis (7,23), that inflammation influences the pathogenesis of diabetes (24) and that systemic inflammation induced in periodontitis may be sufficient to influence the pathogenesis of diabetes (7). Preliminary research has also begun to investigate whether a common inflammatory genetic basis exists between diabetes and periodontal disease (9). Many inflammatory mediators could potentially mediate the relationship between diabetes and periodontal disease. Specifically, high-sensitivity C-reactive protein (hs-CRP), tumour necrosis factor-α (TNF-α), interleukin-1β (IL-1β), interferon-γ (IFN-γ), interleukin-6 (IL-6) and interleukin-18 (IL-18) have all been implicated in the pathogenesis of both diabetes and periodontal disease (18,25–32). However, it is currently unclear whether systemic levels of the above mediators are raised in patients with periodontal disease. Investigations involving patients with aggressive periodontitis in particular are lacking.

Dyslipidaemia is characterized by increased triglycerides and total cholesterol, decreased high-density lipoprotein (HDL)-cholesterol and normal low-density lipoprotein (LDL)-cholesterol, accompanied by trends towards smaller LDL particles. It is accepted that diabetes and dyslipidaemia occur concomitantly (33). Additionally, some studies have supported the existence of dyslipidaemia in patients with periodontitis (34,35). It has been hypothesized that diabetes and periodontal disease may be linked in a cyclic relationship mediated by both lipid and inflammatory mediators; elevated serum lipids in diabetes may predispose to periodontitis by causing a systemic monocytic hyper-responsive trait and, vice versa, periodontitis may exacerbate diabetes via a series of interactions between chronically elevated serum lipids and inflammatory mediators (13). This hypothesis is based on published literature regarding observed relationships between diabetes, periodontitis, dyslipidaemia and inflammation, but some relationships are better established than others. Furthermore, not all research supports the premise that dyslipidaemia is associated with periodontal disease (19) and, regarding dyslipidaemia in patients with aggressive periodontitis, the few studies that have investigated this concept have produced conflicting results (5,36).

There is substantial evidence to support a role for adipokines in metabolic disease, diabetes and the immune response. Evidence has shown that adiponectin acts as an insulin sensitizer (37), whilst leptin reduces insulin secretion (38), and decreased adiponectin and increased leptin concentrations are associated with diabetes (37,39). Evidence also shows that resistin plays a role in insulin resistance, but this is based mainly on animal studies, and the role of resistin in humans is controversial (37). Regarding the immune response, evidence suggests that leptin and resistin play proinflammatory roles, whilst adiponectin exerts anti-inflammatory effects (40). Based on this evidence, it has been proposed that such adipokines may be responsible in part for the associations observed between diabetes and periodontal disease (10,41). However, little is known about the role of these adipokines in periodontal disease. Local levels of leptin have correlated positively with periodontal health (42), whilst increased serum levels of resistin and leptin have been associated with periodontal disease (41,43,44). Serum levels of adiponectin have tended to be reduced in patients with periodontal disease, but not to levels of statistical significance (41,43). Preliminary evidence from *in vitro* studies has suggested that adiponectin may inhibit osteoclast formation (45) and may have an anti-inflammatory effect on host cells in periodontal lesions (46). Conversely, a study in mice found serum adiponectin levels to decrease following inoculation with *Porphyromonas gingivalis* (47), but ligature induction of periodontitis did not affect serum leptin levels in rats (48). Studies in humans have produced some evidence that periodontal treatment may influence serum adipokine levels, but the results of such research have been conflicting (49–51). No evidence is available concerning the roles of these adipokines in aggressive periodontitis.

The aim of this study was therefore to act as a pilot study to investigate glycaemic control and systemic levels of inflammatory mediators, lipids and adipokines in patients with aggressive periodontitis (not previously diagnosed with diabetes) compared with periodontally healthy control subjects.

Material and methods

This pilot study had a case–control design. Levels of random plasma glucose (RPG), HbA_{1c}, hs-CRP, TNF-α, IL-1β, IFN-γ, IL-6, IL-18, triglycerides, total cholesterol, HDL-cholesterol, adiponectin, leptin and resistin were measured in the serum of 30 patients with aggressive periodontitis and 30 age- and sex-matched periodontally healthy control subjects, none of whom had a previous diagnosis of diabetes. This study was granted a favourable ethical opinion by the County Durham and Tees Valley 2 Local Research Ethics Committee, and each study participant provided written informed consent before participating in this study.

Study population

The patients with aggressive periodontitis were recruited from patients referred to Newcastle Dental Hospital (NDH) Periodontology Department, UK, whilst the control subjects with a healthy periodontium were subsequently recruited from patients referred to other departments of NDH or from staff and students working within the hospital.

Inclusion criteria— Participants were all aged 18 years or over and possessed a minimum of 20 teeth.

Aggressive periodontitis was diagnosed according to the prevalence and severity of interproximal bone loss in relation to a patient's age. Patients within defined age ranges (all <45 years old) were required to exhibit a certain level and distribution of interproximal bone loss, assessed radiographically using a dental panoramic radiograph, to be classified as aggressive periodontitis patients. These diagnostic criteria are documented in Table 1 and were adapted from those developed by the UK and Ireland National Periodontal Disease Consortium (unpublished data). Aggressive periodontitis patients all exhibited increased probing depths corresponding to the sites of interproximal bone loss; hence, all demonstrated evidence of current periodontal disease rather than purely historical disease. Alongside the specified interproximal bone loss, patients over 35 years old were also required to demonstrate further aggressive periodontitis characteristics, according to criteria defined at the 1999 World Workshop for the Classification of Periodontal Diseases and Conditions, to ensure that a diagnosis of aggressive periodontitis rather than chronic periodontitis was appropriate. These characteristics included a positive family history (assessed by questioning), microbial deposits inconsistent with the severity of disease and rapid bone destruction (assessed from sequential radiographs). Rapid bone destruction was confirmed by either a significantly large reduction in bone levels relative to the time period between radiographs or by the presence of significant bone loss at 35 years or below.

Table 1. Criteria for the diagnosis of aggressive periodontitis

Age (years)	Number of teeth affected (nonadjacent interproximal sites)	Radiographic interproximal bone loss (mm)
18–19	≥2	≥4
20–24	≥3	≥5
25–29	≥5	≥5
30–34	≥7	≥5
35–45	≥8	≥6

For example, a subject aged 22 years would be required to exhibit radiographic interproximal bone loss of 5 mm or more on three or more teeth (at nonadjacent interproximal sites) to be assigned a diagnosis of aggressive periodontitis. 'Nonadjacent interproximal sites' refers to sites within separate interproximal spaces. Criteria adapted from those developed by the UK and Ireland National Periodontal Disease Consortium (unpublished data).

Periodontally healthy control subjects were age and sex matched to the aggressive periodontitis patients. Age was matched in the following intervals: 18–19, 20–24, 25–29, 30–34, 35–39 and 40–45 years. Periodontally healthy subjects had probing depths of ≤ 3 mm (but up to four probing depths of 4 mm were allowed to account for often increased probing depths at the distal surfaces of last-standing molars) and showed no further clinical or radiographic evidence of current or previous clinical attachment loss or bone loss due to periodontal disease.

Exclusion criteria— The following exclusion criteria were applied: subjects with a known diagnosis of diabetes, pregnant females, subjects with periodontitis as a manifestation of systemic disease, subjects who were immunosuppressed due to disease or medication, subjects with gingival overgrowth as a result of medication, subjects who had any bleeding disorders, took any medications causing prolonged bleeding or had any other medical condition that could compromise their safe participation in the study and subjects who had received periodontal treatment in the past 6 wk.

Data collection

To facilitate the measurement of serum levels of glycaemic control markers, inflammatory mediators, lipid markers and adipokines, a nonfasting blood sample was obtained from each subject either from the antecubital fossa or from the dorsum of the hand using the Vacutainer® system (BD Diagnostics, Franklin Lakes, NJ, USA). Random plasma glucose, HbA_{1c}, triglycerides, cholesterol, HDL-cholesterol and hs-CRP were measured using standard laboratory procedures at the Clinical Biochemistry Department of the Newcastle Royal Victoria Infirmary. Serum levels of TNF-α, IL-1β, IFN-γ and IL-6 were determined via high-sensitivity sandwich immunoassays using MSD® MULTISPOT human cytokine assay ultrasensitive kits (Mesoscale Discovery, Gaithersburg, MD, USA). Serum levels of IL-18, adiponectin, leptin and resistin were determined by ELISA using R&D Systems® Duoset ELISA kits (R&D Systems, Minneapolis, MN, USA). To obtain serum for use in the above cytokine assays and ELISAs, the blood samples were centrifuged for 15 min at 4°C and a relative centrifugal force of 1500*g*, and the serum was subsequently separated and stored in aliquots at −80°C. All samples were run in duplicate.

The following parameters were also recorded: age, sex, ethnicity, medical

history, smoking history and body mass index (BMI). Smoking was assessed according to whether the subjects were current, non- or ex-smokers. The amount smoked by current and ex-smokers was further quantified in pack years (a pack year being equal to smoking one pack of 20 cigarettes per day for 1 year). With respect to BMI, the participants' specific BMIs were calculated from self-reported height and weight values (52), and the participants were classified as underweight (BMI < 18.5 kg/m^2), normal weight (18.5 kg/m$^2 \leq$ BMI < 25 kg/m^2), overweight (25 kg/m$^2 \leq$ BMI < 30 kg/m^2) or obese (BMI ≥ 30 kg/m^2), according to the World Health Organisation (WHO) BMI classification (53).

Statistical analysis

Statistical analyses were conducted using the statistical software SPSS 17 (IBM, Somers, NY, USA). For discrete variables, the numbers and percentages of patients assigned to each category are presented, and differences between the aggressive periodontitis patients and healthy subjects were assessed using chi-squared tests. For continuous variables, normality of the data was assessed using the Kolmogorov–Smirnov test. Means and standard deviations are presented for the parametric variables, whilst medians and interquartile ranges are presented for the nonparametric variables. Differences between the aggressive periodontitis patients and healthy subjects were assessed using Student's unpaired *t*-tests for the parametric variables and Mann–Whitney *U*-tests for the nonparametric variables (unadjusted analyses). Multiple linear regression analyses were also performed, in which the serum mediators were the dependent variables, periodontal status was the independent variable and confounders were BMI, smoking status, ethnicity, age and sex (adjusted analyses). Box plots were also constructed to display the data. Significance in all tests was assessed at the 5% level.

Each immunoassay was run across two plates and therefore interassay variations were calculated for each immunoassay as follows. For each standard (run in triplicate on both plates), its mean concentration reading on each plate was calculated and the average and standard deviation of these two readings found. The value of each standard deviation as a percentage of its corresponding average was calculated, and the mean of these values was quoted as the interassay variation. Lower detection limits were calculated for each immunoassay by multiplying the standard deviation of the zero standard optical densities by 2.5, adding it to the mean of the zero standard optical densities and then plotting this value on the standard curve to produce a concentration reading.

Results

The demographics of the aggressive periodontitis patients and healthy subjects are summarized in Table 2. There were no significant differences between the groups for age, sex, ethnicity, smoking status, pack years smoked (relevant to current and ex-smokers only), BMI or BMI status. All aggressive periodontitis patients were diagnosed as having generalized aggressive periodontitis, according to the criteria defined at the 1999 World Workshop for the Classification of Periodontal Diseases and Conditions (54).

The interassay variations calculated for the leptin, adiponectin, resistin, TNF-α, IL-1β, IL-6, IFN-γ and IL-18 immunoassays were 8.94, 4.19, 4.20, 3.26, 10.52, 2.65, 4.22 and 7.05%, respectively. The lower detection limits calculated for the above immunoassays are illustrated in Fig. 1.

According to unadjusted analyses, there were no significant differences in the RPG, HbA_{1c}, hs-CRP, TNF-α, IL-1β, IL-6, IFN-γ, IL-18, triglyceride, total cholesterol, HDL-cholesterol, adiponectin, leptin or resistin levels between the aggressive periodontitis patients and healthy subjects ($p > 0.05$; Table 3 and Fig. 1). Furthermore, according to adjusted analyses (adjusting for BMI, smoking status, ethnicity, age and sex), there remained no significant differences in

Table 2. Subject demographics

	Aggressive periodontitis ($n = 30$)	Healthy ($n = 30$)	*p*-Value[a]
Sex [*n* (%)]			
Male	11 (36.7)	11 (36.7)	1.000
Age (years)			
[mean (SD)]	36.7 (6.3)	36.3 (6.4)	0.750
Ethnicity [*n* (%)]			
Caucasian	28 (93.3)	29 (96.6)	0.601
Black	1 (3.3)	1 (3.3)	
Asian	1 (3.3)	0 (0)	
Smoking			
status [*n* (%)]			
Current	7 (23.3)	5 (16.7)	0.091
Ex	10 (33.3)	4 (13.3)	
Never	13 (43.3)	21 (70.0)	
Pack years[b]			
[median (interquartile range)]	7.5 (4.1–7.5)	4.0 (3.2–4.0)	0.535
Body mass index (kg/m^2)			
[mean (SD)]	26.0 (3.8)	25.3 (5.0)	0.585
Status [*n* (%)]			
Normal weight	14 (46.7)	15 (50.0)	0.711
Overweight	13 (43.3)	10 (33.3)	
Obese	3 (10.0)	5 (16.7)	

[a] *p*-Values were determined using χ^2 tests for discrete variables, Student's unpaired *t*-tests for continuous parametric variables (body mass index and age) and Mann–Whitney *U*-tests for continuous nonparametric variables (pack years).

[b] Applicable only to current- and ex-smokers ($n = 17$ aggressive periodontitis patients, $n = 9$ healthy subjects).

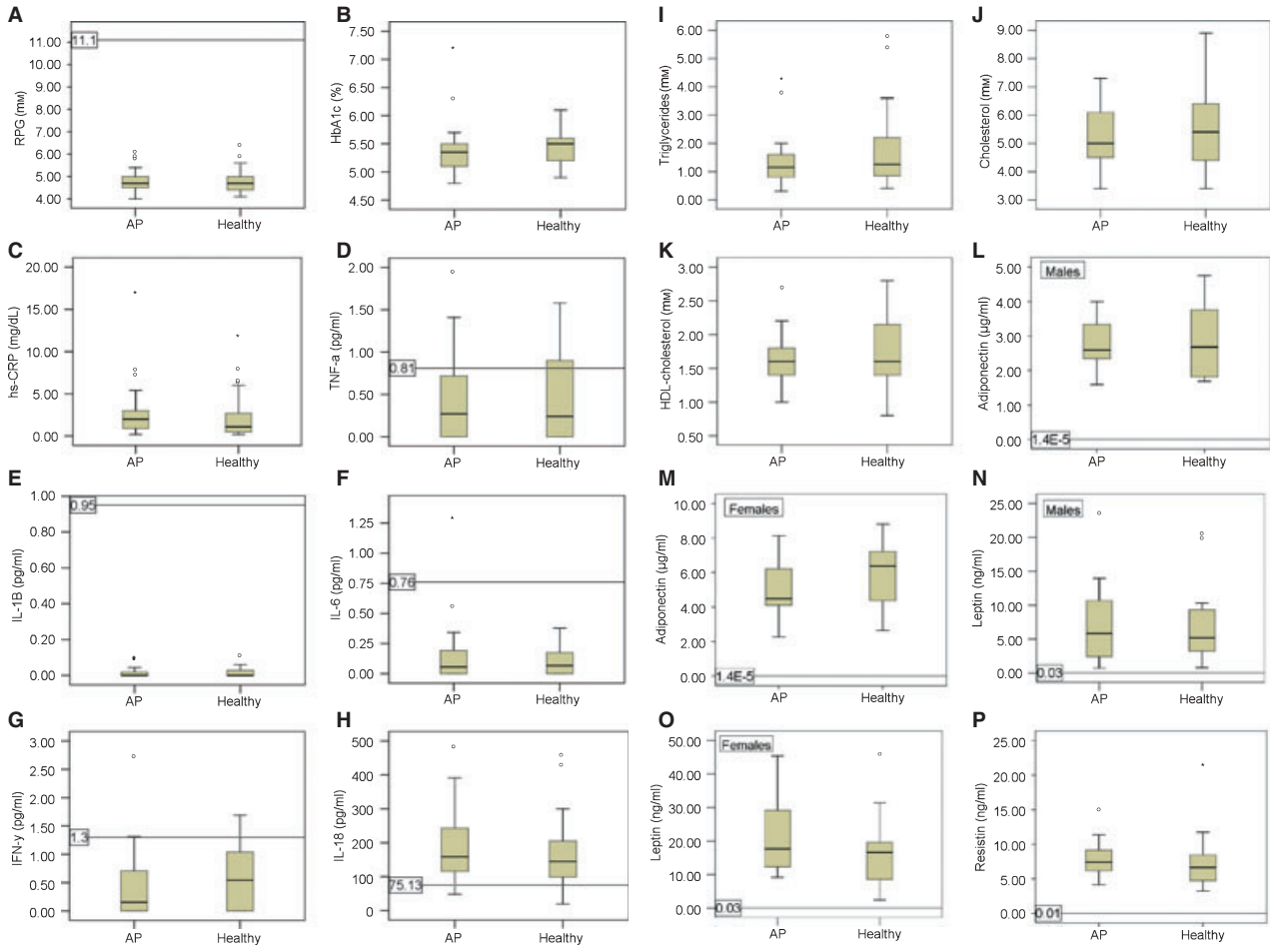

Fig. 1. Box and whisker plots displaying random plasma glucose (RPG; A), glycated haemoglobin (HbA_{1c}; B), high-sensitivity C-reactive protein (hs-CRP; C), tumour necrosis factor-α (TNF-α; D), interleukin-1β (IL-1β; E), interleukin-6 (IL-6; F), interferon-γ (IFN-γ; G), interleukin-18 (IL-18; H), triglyceride (I), cholesterol (J), high-density lipoprotein-cholesterol (K), adiponectin (in males; L), adiponectin (in females; M), leptin (in males; N), leptin (in females; O) and resistin levels (P) in aggressive periodontitis patients and healthy subjects. The median, interquartile and full range of values are shown. Circles represent outliers (values which are between 1.5 and 3 box lengths from the box). Asterisks represent extreme values (values which are more than 3 box lengths from the box). For RPG, the cut-off threshold of 11.1 mM recommended for the diagnosis of diabetes by the WHO and ADA is indicated. For TNF-α, IL-1β, IL-6, IFN-γ, IL-18, adiponectin, leptin and resistin, assay lower detection limits are indicated by labelled horizontal lines.

any of the mediator levels between the aggressive periodontitis patients and healthy subjects ($p > 0.05$; Table 3 and Fig. 1). However, the *p*-values resulting from the unadjusted and adjusted analyses of adiponectin levels in the female patients reached 0.077 and 0.064, respectively, with mean adiponectin levels being reduced in the female aggressive periodontitis patients compared with the female control subjects (mean 4.94 and 5.97 µg/mL, respectively). The RPG levels were all below 11.1 mM, which is the diagnostic threshold value recommended by the WHO and an Expert Committee sponsored by the American Diabetes Association (ADA) when RPG is used to diagnose diabetes (55,56).

Discussion

The aim of this pilot study was to investigate glycaemic control and systemic levels of inflammatory mediators, lipids and adipokines in patients with aggressive periodontitis (not previously diagnosed with diabetes) compared with periodontally healthy control subjects. This study provided no evidence to suggest that serum levels of HbA_{1c}, RPG, hs-CRP, TNF-α, IL-1β, IFN-γ, IL-6, IL-18, triglycerides, total cholesterol, HDL-cholesterol, adiponectin, leptin and resistin are altered in nondiabetic patients with aggressive periodontitis compared with periodontally healthy control subjects. Notably, this was the first study to investigate serum levels of HbA_{1c}, IL-18, leptin, adiponectin and resistin in subjects with aggressive periodontitis.

The finding that there was no difference in the levels of RPG and HbA_{1c} between the aggressive periodontitis patients and periodontally healthy subjects does not provide any evidence to suggest that patients with aggressive periodontitis have evidence of diabetes. This was hypothesized based on previous epidemiological evidence

Table 3. Levels of metabolic and inflammatory markers

	Aggressive periodontitis (n = 30)	Healthy (n = 30)	p-Value (unadjusted)	p-Value (adjusted)
Random plasma glucose (mM)	4.80 (5.24)	4.78 (5.12)	0.921	0.628
Glycated haemoglobin (%)	5.35 (5.10–5.53)	5.50 (5.20–5.63)	0.201	0.398
Triglycerides (mM)	1.15 (0.78–1.63)	1.25 (0.83–2.20)	0.548	0.246
Cholesterol (mM)	5.24 (1.09)	5.51 (1.39)	0.410	0.173
High-density lipoprotein-cholesterol (mM)	1.61 (0.35)	1.71 (0.52)	0.408	0.170
High-sensitivity C-reactive protein (mg/dL)	2.00 (0.78–3.08)	1.10 (0.50–2.75)	0.359	0.248
Tumour necrosis factor-α (pg/mL)	0.27 (0.00–0.72)	0.24 (0.00–0.90)	0.940	0.979
Interleukin-1β (pg/mL)	0.00 (0.00–0.02)	0.00 (0.00–0.03)	0.826	0.766
Interleukin-6 (pg/mL)	0.06 (0.00–0.19)	0.06 (0.00–0.18)	0.869	0.507
Interferon-γ (pg/mL)	0.16 (0.00–0.72)	0.45 (0.00–1.04)	0.175	0.548
Interleukin-18 (pg/mL)	158.66 (114.76–245.18)	144.80 (89.72–205.15)	0.495	0.805
Leptin (males) (ng/mL)	7.62 (6.90)	7.79 (6.76)	0.954	0.626
Leptin (females) (ng/mL)	21.53 (11.52)	16.58 (10.63)	0.177	0.124
Adiponectin (males) (μg/mL)	2.79 (0.74)	2.94 (1.11)	0.713	0.803
Adiponectin (females) (μg/mL)	4.94 (1.56)	5.97 (1.93)	0.077	0.064
Resistin (ng/mL)	7.40 (6.15–9.21)	6.65 (4.71–8.60)	0.198	0.561

Means and SDs are presented for parametric variables [random plasma glucose, cholesterol, high-density lipoprotein-cholesterol, leptin (males), leptin (females), adiponectin (males) and adiponectin (females)]. Medians and interquartile ranges are presented for nonparametric variables (all other variables). Unadjusted p-values were determined using Student's unpaired t-tests for parametric variables and Mann–Whitney U-tests for nonparametric variables. Adjusted p-values are the results of multivariate linear regression analyses adjusting for body mass index, smoking status, ethnicity, age and sex.

linking diabetes with periodontal disease, including aggressive periodontitis (2,3,5,8). The only previous study investigating glycaemic control in nondiabetic subjects with aggressive periodontitis measured RPG levels in patients with aggressive periodontitis or severe chronic periodontitis and found these to be significantly increased in this combined 'severe periodontitis' subject group compared with periodontally healthy control subjects (5). That study, however, does not appear to directly compare glucose levels of aggressive periodontitis patients alone with those of healthy control subjects and also does not account for BMI as a confounding factor. Previous studies measuring fasting plasma glucose levels and HbA_{1c} levels in nondiabetic patients with less severe forms of periodontal disease also found these markers to be significantly raised in such patients (3,4).

Although all of the RPG levels were well below the threshold of 11.1 mM recommended for a diagnosis of diabetes (55,56) and therefore reveal no evidence of diabetes in any of the subjects, it is worth noting that two aggressive periodontitis patients but no healthy subjects had HbA_{1c} levels above 6.1% and that the level in one such aggressive periodontitis patient exceeded 7%. Glycated haemoglobin is not currently recognized as a diagnostic indicator for diabetes (57), but the above values have been proposed by some researchers as suitable threshold levels for screening or diagnostic tests for diabetes (58,59). Interestingly, the RPG levels in the above two aggressive periodontitis patients were also elevated, being in the top 10% of all RPG measurements. The proportions of patients with HbA_{1c} levels above 6.1% were not significantly different between the aggressive periodontitis and healthy groups according to a chi-squared analysis (p = 0.150). Hence, this may be purely a chance finding; however, further investigation may be warranted.

We acknowledge that HbA_{1c} is not a recognized diagnostic indicator for diabetes (57) and that, although the use of RPG is endorsed for clinical diagnoses, it is not recommended for the diagnosis of diabetes for epidemiological purposes (55). The WHO and International Diabetes Federation recommend the use of either a single fasting plasma glucose test or an oral glucose tolerance test for the diagnosis of diabetes for epidemiological purposes (55,57). However, patient co-operation issues precluded the use of the above tests in this study. The concern is that RPG measurements may be affected by recent food intake, and there are concerns about the availability and precision of the HbA_{1c} measurement globally (57). However, the WHO and International Diabetes Federation do state that, in reference laboratories, the precision of the HbA_{1c} measurement is similar to that of the fasting plasma glucose test and oral glucose tolerance test (57).

This study provides no evidence to suggest that patients with aggressive periodontitis have an altered systemic inflammatory profile, with respect to serum hs-CRP, TNF-α, IL-1β, IFN-γ, IL-6 and IL-18. Previously, it has been proposed that local inflammatory cytokines and/or bacteria and their products may leak into the systemic circulation in periodontal disease and induce an inflammatory state and also that periodontitis may be a manifestation of a heightened systemic inflammatory state, whether due to genetics or systemic disease, and both such theories have been advocated as possible contributors to the association between diabetes and periodontal disease (7,10,13,15,60). Substantial evidence has previously demonstrated

circulating CRP levels to be raised in aggressive periodontitis patients (21,61). However, no difference has been reported in serum levels of IL-1β and IFN-γ between aggressive periodontitis patients and periodontally healthy subjects (62,63). Both elevated and normal levels of serum TNF-α and IL-6 have been associated with aggressive periodontitis (61–63), whilst no data are available concerning serum levels of IL-18 in aggressive periodontitis patients. In patients with nonaggressive forms of periodontal disease, elevated serum CRP levels have been repeatedly reported (3), elevated serum TNF-α, IL-1β, IL-6 and IL-18 levels have been reported but not ubiquitously (3,19,64–67), and no difference has been reported in serum levels of IFN-γ between such subjects and periodontally healthy control subjects (3). Conflicting results between studies are most probably due to variations in study methods, such as the selection of study participants, clinical and laboratory analytical techniques employed and the measurement of confounding factors.

Our findings that serum triglyceride, total cholesterol and HDL-cholesterol levels were no different between patients with aggressive periodontitis and periodontally healthy control subjects does not support the hypothesis that patients with aggressive periodontitis have altered serum lipid levels. This has been postulated based on preliminary evidence suggesting that periodontitis may induce dyslipidaemia and that dyslipidaemia may predispose to periodontitis (68) and the hypothesis that dyslipidaemia may mediate the association between diabetes and periodontal disease (13). Only two previous studies have investigated lipid levels in patients with aggressive periodontitis, and the results were conflicting. Nibali *et al.* (5) found significant differences in LDL-cholesterol and HDL-cholesterol levels between patients with either aggressive periodontitis or severe chronic periodontitis and periodontally healthy control subjects, but no significant differences in total cholesterol and triglyceride levels between these groups. In contrast, Rufail *et al.* (36,69) found no difference in LDL-cholesterol and HDL-cholesterol levels between patients with generalized aggressive periodontitis, localized aggressive periodontitis and periodontally healthy control subjects, but found significantly raised total cholesterol and triglyceride levels in patients with generalized aggressive periodontitis compared with patients with localized aggressive periodontitis and healthy control subjects. It is important to note that subject numbers were limited in the latter study (12 generalized aggressive periodontitis patients and 12 localized aggressive periodontitis patients) and, in the former study, the aggressive periodontitis subjects alone were not directly compared with the periodontally healthy subjects and BMI was not accounted for as a confounding factor. Both normal lipid profiles and elevated levels of dyslipidaemia have been reported in subjects with nonaggressive forms of periodontal disease (19,70).

To the best of our knowledge, this is the first study to measure adiponectin, leptin and resistin in the serum of patients with aggressive periodontitis. It provides no evidence to support the hypothesis that serum levels of such adipokines are altered in aggressive periodontitis patients. This hypothesis was based on preliminary evidence supporting the existence of a relationship between periodontal disease and adipokines (41,46,47), which also influenced the theory that adipokines may play a role in inter-relationships between diabetes and periodontal disease (10,41). The authors would like to highlight that the *p*-values resulting from the unadjusted and adjusted analyses of adiponectin levels in the female patients were 0.077 and 0.064, respectively. Being only slightly outside the 5% significance level, this may be suggestive of a tendency towards decreased adiponectin levels in the aggressive periodontitis patients. Such a tendency would concord with previous evidence supporting an anti-inflammatory role for adiponectin in the immune response (40). With specific regard to periodontal disease, it would be in accordance with preliminary *in vitro* evidence suggesting that adiponectin may inhibit osteoclast formation (45) and may have an anti-inflammatory effect on host cells in periodontal lesions (46) and also with findings that serum adiponectin levels decrease in mice following inoculation with *Porphyromonas gingivalis* (47). Previous studies have found patients with nonaggressive forms of periodontal disease to have increased serum levels of resistin and leptin (41,43,44) and a tendency towards reduced adiponectin levels (41,43). Again, the latter have not reached levels of statistical significance. It is evident that significantly more research will be required before the roles of adipokines in periodontal disease and aggressive periodontitis are elucidated. Leptin and adiponectin levels were analysed separately for male and female subjects because these levels were significantly higher in the female subjects than in the male subjects ($p = 0.000$).

The strengths of the present study were that multiple serum mediators were measured simultaneously and that confounding factors were accounted for, both by matching procedures and statistical analyses. Random plasma glucose, HbA_{1c}, hs-CRP, triglyceride, total cholesterol and HDL-cholesterol were measured at the NHS Clinical Biochemistry Department of the Newcastle Royal Victoria Infirmary according to national quality control standards. For the immunoassays run to measure TNF-α, IL-1β, IL-6, IFN-γ, IL-18, adiponectin, leptin and resistin, low inter-assay variations were obtained. However, although the adiponectin, leptin and resistin levels measured were all above the calculated lower detection limits of their respective immunoassays, the majority of the TNF-α, IL-1β, IL-6 and IFN-γ levels and a small number of the IL-18 levels measured were below the calculated lower detection limits of their corresponding immunoassays (Fig. 1). Furthermore, this pilot study was limited by small sample sizes. This is a frequent problem in studies of aggressive periodontitis, which occurs infrequently in the population. In this pilot study, a sample size of 30 patients in each group was selected as an achievable sample which would be sufficient to inform power calculations for the

determination of sample size in future studies. For example, based on the adiponectin levels observed in the female patients in this study, it can be calculated that 61 female aggressive periodontitis subjects and 61 female healthy subjects would be required to detect this difference at the 5% significance level with a power of 90%.

Finally, the criteria employed to diagnose aggressive periodontitis in this study must be discussed. A multitude of criteria have been employed to diagnose aggressive periodontitis in previous research studies, but many studies have based their diagnoses on the criteria defined at the 1999 World Workshop for the Classification of Periodontal Diseases and Conditions, which distinguished the primary three features of aggressive periodontitis as systemic health, rapid attachment loss/bone destruction and familial aggregation (54). The above criteria, however, can be subjective, and it is often difficult to identify the latter two characteristics because previous clinical records detailing changes in disease levels over time or reliable information regarding the periodontal history of family members are often not available. For these reasons, we used a classification system which diagnosed aggressive periodontitis based on the prevalence and severity of periodontal bone loss in relation to age. A recent review by Demmer and Papapanou (71) supports the above concerns regarding the 1999 Classification criteria, and the authors conclude that, although appropriate for clinical diagnoses, the above criteria are often not appropriate for research purposes. These authors support a classification system for the diagnosis of aggressive periodontitis in research studies based on an assessment of the loss of periodontal tissue in relation to age. Unfortunately, however, as our study was undertaken prior to the publication of the report by Demmer and Papapanou, our aggressive periodontitis classification criteria do differ from their recommended criteria. Demmer and Papapanou (71) also exclude patients above the age of 35 years from their inclusion criteria because they suggest that aggressive periodontitis can only be diagnosed in such patients by access to disease progression data and/or confirmation of familial aggregation. We included patients up to the age of 45 years in our aggressive periodontitis group, with 21 of the aggressive periodontitis patients being over 35 years old. However, such patients over 35 years old were required to demonstrate further aggressive periodontitis characteristics (a positive family history, microbial deposits inconsistent with the severity of disease and rapid bone destruction), in addition to the specified interproximal bone loss criteria, to ensure that a diagnosis of aggressive periodontitis rather than chronic periodontitis was appropriate. However, as discussed above, we recognize the disadvantages associated with these 1999 classification criteria; they are open to subjective interpretation and are often subject to a lack of available or reliable information. Specifically, we acknowledge that sequential radiographs were not available for every patient over 35 years of age. However, a diagnosis of aggressive periodontitis was only made if there was sufficient evidence to support a diagnosis when sequential radiographs, family history and the presence of microbial deposits in relation to the severity of disease were all considered in conjunction. Furthermore, we acknowledge that clinical judgement was applied to determine how much bone loss constituted a significantly large reduction in bone levels relative to the time period between radiographs or the presence of significant bone loss at 35 years or below, and that this is subjective. However, there are unfortunately no widely accepted criteria available to provide guidance regarding what constitutes rapid bone destruction in aggressive periodontitis. In their systematic review published in 2002, Mombelli *et al*. (72) present criteria for defining 'secure', 'uncertain' and 'insecure' diagnoses of generalized aggressive periodontitis. Their criteria incorporate very specific thresholds with regard to attachment loss, but thresholds for bone loss are not defined with the same level of precision. It is for these exact reasons that we primarily base our diagnoses of aggressive periodontitis in this study on the specific criteria regarding radiographic interproximal bone loss in Table 1. We did repeat the statistical analyses for subjects of 35 years or less. According to unadjusted analyses, IFN-γ was significantly higher in the healthy patients compared with the aggressive periodontitis patients ($p = 0.042$). However, according to adjusted analyses, there were no significant differences in any serum mediator levels between the aggressive periodontitis patients and healthy control subjects ($p > 0.05$).

In conclusion, within the limits of this pilot study, there was no evidence to suggest that patients with aggressive periodontitis, not previously diagnosed with diabetes, have evidence of diabetes or altered serum levels of the above inflammatory mediators, lipids and adipokines. In particular, we acknowledge that the lack of associations may be due to a lack of power and, therefore, we recommend that this pilot study be used to inform future research incorporating larger sample sizes.

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