J Clin Periodontol 2006; 33: 784–790 doi: 10.1111/j.1600-051X.2006.00984.x

Journal of Clinical Periodontology

The influence of diabetes on gingival crevicular fluid β-glucuronidase and interleukin-8

Steven P. Engebretson[1], Faranak Vossughi[2], Judith Hey-Hadavi[2,3], Gülnur Emingil[4] and John T. Grbic[2]

[1]Department of Periodontics, School of Dental Medicine, Stony Brook University, Stony Brook, NY, USA; [2]Columbia University College of Dental, Medicine, Division of Periodontics, New York, NY, USA; [3]Pfizer Inc., New York, NY, USA; [4]Department of Periodontology, School of Dentistry, Ege University, Izmir, Turkey

Engebretson SP, Vossughi F, Hey-Hadavi J, Emingil G, Grbic JT. The influence of diabetes on gingival crevicular fluid β-glucuronidase and interleukin-8. J Clin periodontol 2006; 33: 784–790. doi: 10.1111/j.1600-051X.2006.00984.x.

Abstract

Objectives: Polymorphonuclear neutrophil (PMN) dysfunction is associated with diabetes. We examined the gingival crevicular fluid (GCF) β-glucuronidase (BG) and interleukin-8 (IL-8) levels of periodontitis patients with and without type 2 diabetes mellitus (DM).

Material and methods: Forty five adults with type 2 DM and 32 adults without DM, both with chronic periodontitis were enrolled. GCF was collected from eight posterior sites in each quadrant, and periodontal parameters were recorded. GCF was assayed for IL-8 by ELISA and BG by a flourometric assay.

Results: GCF IL-8 was positively correlated with probing depth (PD), and GCF BG but not clinical attachment level (CAL), bleeding on probing (BOP), or plaque index (PI). In contrast, GCF BG was strongly correlated with each of the clinical measures of periodontal disease. Subjects with DM significantly lower levels of both BG (73.0 ± 44.8 *versus* 121.9 ± 84.6 pg/sample; $p = 0.002$) and IL-8 (32.1 ± 33.1 *versus* 90.8 ± 83.2 pg/sample; $p < 0.0001$) even after adjustments for age, gender, PD, CAL, BOP, and PI. Neither BG nor IL-8 was correlated with HbA1c levels in subjects with DM.

Conclusion: These data suggest that an inadequate local response by PMN, partially explained by an altered chemokine gradient, may contribute to periodontal disease in patients with type 2 DM.

Key words: adult; β-glucuronidase; diabetes mellitus; gingival crevicular fluid; HbA1c; interleukin-8; periodontitis; PMN

Accepted for publication 5 July 2006

Individuals with type 2 diabetes mellitus (DM) have greater incidence and severity of periodontal disease than individuals without DM of similar age (Tervonen & Knuuttila 1986, Oliver & Tervonen 1993, Taylor et al. 1996, Tsai et al. 2002). While the pathogenesis of diabetes-related periodontitis is not fully defined (Soskolne & Klinger 2001), polymorphonuclear neutrophil (PMN) chemotaxis (Iacono et al. 1985), phagocytosis (Delamaire et al. 1998), oxidative burst (Karima et al. 2005), and killing ability (Sawant 1993) have been reported to be altered in the hyperglycemic state (Esposito et al. 2002). Given the important role of the PMN in periodontal homeostasis (Lamster & Novak 1992), an altered or impaired PMN function may partially explain the increased susceptibility to periodontitis experienced by people with diabetes. However, the evidence for an altered PMN functionality in diabetes has been inconsistent. Diminished (Kjersem et al. 1988, Noritake et al. 1992, Sawant 1993), normal (McManus et al. 2001), or exuberant (Noack et al. 2000, Karima et al. 2005) PMN function have both been reported. This inconsistency of findings can partially be explained by noting that these studies examine different aspects of PMN function e.g. chemotaxis, respiratory burst, lysosomal enzyme release, and phagocytosis (killing function) (McManus et al. 2001).

Diminished killing capacity of PMN derived from DM subjects may be due to defective PMN chemotaxis (Mowat & Baum 1975, Iacono et al. 1985, Cutler et al. 1991, Delamaire et al. 1998) or release of PMN granules as reported by Muchova et al. (1999). Hyperglycemia leads to increased PMN superoxide production (Lin et al. 1995, Karima et al. 2005), and oxidative stress in the diabetic state may augment existing chronic inflammatory diseases, such as periodontitis (Schmidt et al. 1996), asthma (Lin et al. 1995), and cardiovascular disease (Schmidt et al. 1994). This observation was recently confirmed and expanded by Karima et al. (2005) in a report that also suggests a mechanism whereby hyperglycemia leads to elevated protein kinase C activity resulting in increased phosphorylation of p47-*phox* and O2 release.

The role of PMN phagocytosis and killing in periodontitis associated with

diabetes is less clear. Primary granule contents of subjects with DM appear to be similar to those of controls without DM (Sawant 1993) but granule release is diminished (Advani et al. 2002, Collison et al. 2002). In spite of enhanced superoxide production by PMN from subjects with DM, reduced bactericidal activity has been observed (Kjersem et al. 1988, Gallacher et al. 1995, Collison et al. 2002). PMN from subjects with DM have been noted to have increased adherence to endothelium, and spontaneous activation (Delamaire et al. 1998), that may render them ineffective to a microbial challenge. Advani et al. (2002) have proposed a mechanism for defective granule release in PMN from subjects with DM whereby defective actin polymerization leads to persistent CD11b expression.

β-Glucuronidase (BG) is an acid hydrolase released by primary granules of PMN in response to stimuli such as *N*-formyl–methionyl–leucyl–phenylalanine, platelet-activating factor, anaphylotoxin C5a, leukotriene B_4, and interleukin-8 (IL-8) (Harper et al. 1989). Increased levels of BG in gingival crevicular fluid (GCF) have been associated with increased risk for periodontal attachment loss in systemically healthy individuals (Lamster et al. 1995, Grbic et al. 1999), and are strongly correlated with probing depth (PD). To date, only one study has evaluated BG in the GCF of subjects with diabetes. Oliver and colleagues found that increased BG levels were associated with poor glycemic control (Oliver & Tervonen 1993). However, that study did not control for PD at the sampled site (Lamster et al. 1991), which is a strong predictor of GCF BG. Hence, it remains unclear whether diabetes patients with periodontitis are qualitatively different with regards to levels of GCF BG.

IL-8 is a chemokine important for PMN recruitment and also for PMN priming. IL-8 transcript has been shown histologically to be tightly linked to the recruitment of PMN to the gingival sulcus (Tonetti et al. 1994). IL-1 induces IL-8, and GCF IL-8 is known to be correlated with IL-1 (Payne et al. 1993), and with periodontal clinical parameters (Tsai et al. 1995, Giannopoulou et al. 2003). A deficient or ineffective chemokine gradient may impair PMN response to pathogens and confer increased susceptibility to periodontitis (Mathur et al. 1996, Chung et al. 1997, Ozmeric et al. 1998, Gainet et al. 1999, Figueredo & Gustafsson 2000, Gamonal et al. 2000). For example, IL-8 secretion is impaired in the urine of women with DM and asymptomatic bacteriuria compared with women without DM (Geerlings et al. 2000). Interestingly, urinary tract infections are also more prevalent in patients with diabetes. Hence, an altered chemokine gradient in the GCF of DM patients with periodontitis might result in a diminished PMN barrier function and lead to increased attachment loss. To date however, there is little information regarding markers of PMN function in the GCF of periodontitis patients with type 2 DM.

The purpose of this study was to examine and compare the GCF BG and IL-8 levels of patients with similar chronic periodontitis severity, with and without type 2 DM.

Materials and Methods

Patient selection

Forty-five subjects with type 2 DM and 32 subjects without diabetes took part in this cross-sectional study. All subjects had a diagnosis of moderate to severe periodontitis. The enrollment criteria were as follows: at least 20 teeth; two sites of $\geqslant 5$ mm clinical attachment loss in each quadrant; no antibiotic usage within 6 months; not a regular user of non-steroidal anti-inflammatory drugs (NSAIDs). A subject was considered a non-smoker if he or she had never smoked, or had stopped smoking more than 5 years previous to the date of examination and had a pack-year history of less than 10. Additional exclusion criteria were: pregnancy or lactation, HIV infection, bleeding disorders, or immunosuppressive chemotherapy.

This study was approved by the Institutional Review Board at Columbia Presbyterian Medical Center. Informed consent was obtained from all subjects.

GCF Collection

The clinical evaluation was preceded by collection of GCF as previously described from the mesiolingual and mesiobuccal surfaces of the first molar tooth in each quadrant (Lamster et al. 1991). If the first molar was absent, the second molar was sampled. If both the first and second molar were missing the second pre-molar was sampled. If there were no posterior teeth in a quadrant no sample was taken from that quadrant. Briefly, teeth were air-dried and isolated with cotton rolls, supragingival plaque was gently removed, and GCF was sampled with pre-cut methylcellulose filter paper strips for 30 s. Strips were measured for fluid volume with a calibrated Periotron 6000 (Interstate Drug Exchange, Amityville, New York, USA), then removed to separate microcentrifuge tubes containing 50 μL phosphate-buffered saline-Tween 20. The tubes were stored at -20°C until eluted (maximum 48 h). Following elution, each GCF sample was analysed separately.

Clinical measures

Clinical data included PD, clinical attachment level (CAL), supragingival plaque, and bleeding on probing (BOP) and were collected at six sites per tooth. PD was defined as the distance in millimetres from the coronal-most margin of the free gingiva to the most apical penetration of the Michigan-O probe. CAL was defined as the distance from the cemento-enamel junction to the most apical penetration of the Michigan-O probe. The presence of supragingival plaque was recorded dichotomously during PD measurements. BOP within 20 s was also recorded dichotomously.

Analysis of GCF samples for IL-8 and BG

GCF samples were analysed for IL-8 using a commercially available enzyme-linked immunosorbant assay (R&D Systems, Minneapolis, MN, USA). This assay is a sandwich ELISA and was performed according to manufacturer's instructions using human recombinant standards. Results are reported as total amount of IL-8 (in pg $\pm$ SD) per 30 s sample, and concentration as described previously (Lamster et al. 1996, Engebretson et al. 1999). Levels of BG were measured as previously described (Lamster et al. 1991). Briefly, BG is measured by using a fluorometric assay utilizing a synthetic substrate that emits fluorescence when cleaved by BG. The assay is highly specific for mammalian BG and the results are expressed as fluorescence units/sample.

Where BG and IL-8 levels are compared between patient groups, mean whole-mouth values are used. Where mediator levels are reported by PD category, mean values are calculated for each PD category for each patient.

Statistical analysis

Means, median and inter-quartile ranges were calculated. Comparisons for continuous variables were made with parametric and non-parametric tests. Categorical variables were compared with tests of proportions. Linear regression models were fit to predict GCF BG and IL-8.

Results

Clinical parameters

Of the 77 subjects, 43 (51.5%) were females. The mean age was 48.7 years with a range of 25–69 years. Subjects with diabetes were older than patients without DM (mean age 54.5 *versus* 40.6 years; $p = 0.01$), otherwise there were no significant differences between groups. The two groups were similar with regards to clinical periodontal parameters. Mean GCF volume was higher in non-diabetic subjects (145.4 ± 38.1 *versus* 117.5 ± 30.7 Periotron units/30 s sample, $p = 0.003$, Mann–Whitney test). In order to test whether GCF volume was a determining factor for BG and IL-8, we used log-transformed values in an ANOVA model. PD of the sampled site and DM status, but not GCF volume, were strong predictors of BG and IL-8, adjusting for age and sex (not shown). We, therefore, used total amount of analytes in the analysis. Demographic and clinical parameters of the individuals in this study (mean ± standard error) are provided in Table 1.

Table 1. Characteristics of participants

Characteristic *N* (%) or mean ± SD (unless otherwise indicated)	DM 45	Non-DM 32	*p*
Age in years	54.2 ± 9.3	42.0 ± 10.6	0.001
% female	54	47	0.51
% current smoker	19	16	
% former smoker	16	27	
Mean whole-mouth attachment loss (mm)	4.0 ± 1.3	3.6 ± 0.9	0.07
Mean whole-mouth probing depth (mm)	3.3 ± 0.8	3.3 ± 0.7	0.81
% BOP	56.1	55.8	0.96
% plaque	77.1	69.4	0.12
Mean GCF BG (fluorescent units/sample)	70.7 ± 43.7	121.9 ± 84.6	0.001
Mean GCF IL-8 (pg/sample)	33.0 ± 33.4	90.8 ± 83.2	<0.00001

GCF, gingival crevicular fluid; BG, β-glucuronidase; BOP, bleeding on probing; IL-8, interleukin-8.

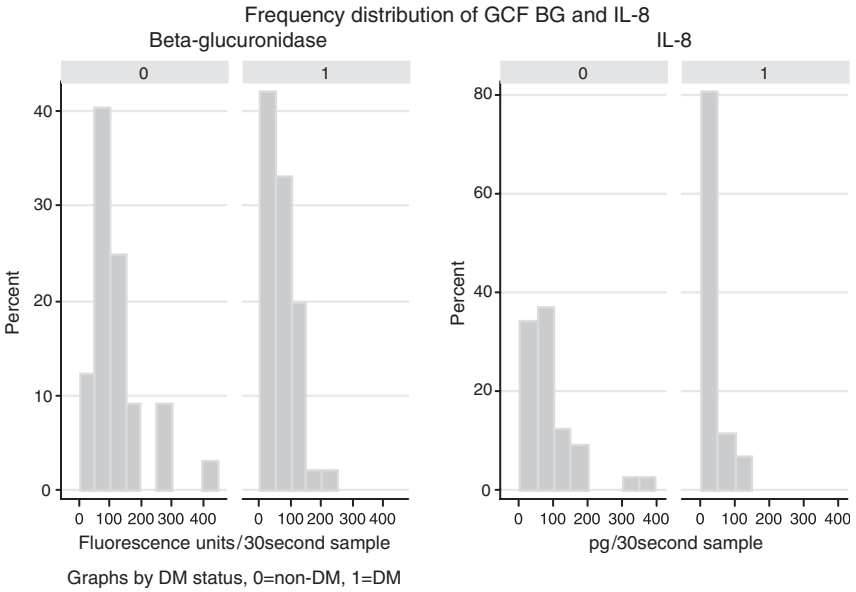

Fig. 1. Frequency distribution of mean subject gingival crevicular fluid (GCF) β-glucuronidase and interleukin-8 (IL-8) levels by diabetes status.

BG activity

BG activity was present in all sites tested (range 12.5–408.7 fluorescent units/30 s sample) and followed a skewed distribution (Fig. 1). As has been shown previously, BG was significantly correlated with PD of the sampled site (spearman's rank correlation, $p < 0.0001$) regardless of diabetes status. Also, mean BG levels were significantly correlated with mean CAL ($\rho = 0.44$, $p = 0.0001$), PD ($\rho = 0.56$, $p < 0.00001$), %BOP ($\rho = 0.47$, $p < 0.00001$), but not plaque index (PI) ($\rho = 0.22$, $p = 0.06$). The correlations between periodontal clinical parameters and BG were seen regardless of diabetes status (not shown). Mean GCF BG activity levels were significantly lower in subjects with DM compared those without DM (Mann–Whitney, $p = 0.001$) (Fig. 2). This was true regardless of attachment loss severity (Fig. 2). In a linear regression model with normalized GCF BG as the dependent variable, diabetes status and periodontal disease severity were both strong predictors for BG adjusting for age and gender (Table 2).

In contrast with BG, mean GCF IL-8 levels were significantly correlated with mean CAL (Spearman's $\rho = 0.40$, $p = 0.02$), and PD ($\rho = 0.41$, $p = 0.02$) among subjects without DM only. For DM subjects, there was no association for GCF IL-8 and CAL ($\rho = 0.21$, $p = 0.18$), PD ($\rho = 0.28$, $p = 0.07$), %BOP ($\rho = -0.0009$, $p = 0.99$), or PI ($\rho = -0.13$, $p = 0.40$). GCF IL-8 levels were significantly lower in DM subjects compared with subjects without DM (Mann–Whitney, $p < 0.00001$) (Fig. 3). As with BG, GCF IL-8 levels were lower in subjects with DM regardless of periodontitis severity, or PD of the site sampled.

In a linear regression model with normalized GCF IL-8 as the dependent variable, only diabetes status and periodontal disease severity were significant predictors of IL-8 (adjusting for age, gender, and BG).

Among all subjects BG and GCF IL-8 were weakly correlated ($\rho = 0.22$, $p = 0.04$) with each other. Neither BG ($\rho = 0.21$, $p = 0.17$) nor IL-8 ($\rho = -0.07$, $p = 0.67$) was associated with metabolic control among DM subjects.

Discussion

The major finding of the present study was that GCF levels of the PMN chemokine IL-8, and lysosomal enzyme BG were significantly lower among adult

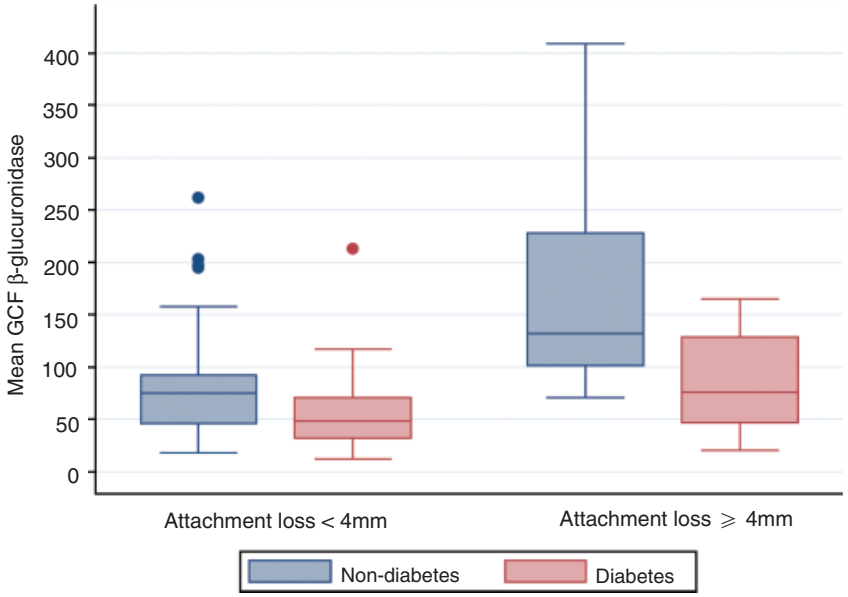

Fig. 2. Gingival crevicular fluid (GCF) *β*-glucuronidase by attachment loss category in subjects with and without type 2 diabetes.

Table 2. Linear regression models for GCF *β*-Glucuronidase and IL-8

	Coefficient (SE)
Log (*β*-Glucuronidase)	
Age (years)	0.00444 (0.0067)
Sex (female)	0.278** (0.13)
CAL > 4 mm	0.514*** (0.14)
DM Status	−0.633*** (0.16)
Constant	4.108*** (0.30)
R^2	0.32
Log (IL-8)	
Age	0.000215 (0.0099)
Sex	−0.00358 (0.20)
CAL > 4 mm	0.598*** (0.23)
DM Status	−1.196*** (0.26)
Log BG	−0.141 (0.17)
Constant	4.629*** (0.84)
R^2	0.37

*$p < 0.1$,
**$p < 0.05$,
***$p < 0.01$.
[]CAL > 4 mm, mean attachment loss greater than 4 mm; DM status, diabetes mellitus; LogBG, *β*-Glucuronidase; standard errors in parentheses.

subjects with type 2 DM compared with subjects without DM of similar periodontal disease severity. GCF volume did not account for these differences. GCF BG was correlated with periodontal clinical parameters in subjects with and without DM, but the total amount of BG was significantly lower in DM subjects regardless of PD. Significant correlations were observed between GCF IL-8 and clinical periodontal parameters only in subjects without DM. Taken together, these data suggest a diminished PMN response in the gingival sulcus of DM individuals with chronic periodontitis that may be partially explained by an altered or inadequate chemokine gradient.

There have been a number of reports of IL-8 in the GCF but to our knowledge the present study is the first to report IL-8 in the GCF of DM subjects. The finding of lower levels of IL-8 in the GCF of DM individuals was unexpected, as diabetes is often associated with increased inflammatory mediators, such as IL-1*β* (Salvi et al. 1998, Engebretson et al. 2004) that is known to induce IL-8 expression (Payne et al. 1993).

In contrast to GCF, circulating IL-8 has been shown in several studies to be elevated in subjects with DM compared with controls (Zozulinska et al. 1999, Herder et al. 2005). Hyperglycemia is associated with increased inflammatory cytokines in the serum (Esposito et al. 2002), and IL-8 transcript is induced in endothelial cells in the presence of excess glucose (Srinivasan et al. 2003). A lower extracorporeal IL-8 was proposed by Geerlings et al. (2000) to account for increased urinary tract infections in women with DM. Thus, our finding of diminished IL-8 levels in the GCF of diabetic subjects is not without precident. IL-8 is important for PMN recruitment (Tonetti et al. 1994) and also promotes monocyte adhesion (Srinivasan et al. 2003). A diminished host response due to low GCF IL-8 levels was suggested by Mathur et al. (1996) to account for increased susceptibility to attachment loss. Alternatively, the PMN activity of individuals with DM may be inherently diminished. The latter explanation would be consistent with Karima et al. (2005) who demonstrated enhanced superoxide production in PMN from subjects with DM. While the precise mechanism for lower GCF IL-8 in subjects with DM is unknown, a deficient or ineffective chemokine gradient may impair PMN response to pathogens and confer increased susceptibility to periodontitis (Mathur et al. 1996, Chung et al. 1997, Ozmeric et al. 1998, Gainet et al. 1999, Figueredo & Gustafsson 2000, Gamonal et al. 2000).

Diminished PMN activity in the gingival sulcus is seemingly at odds with the long-established finding that BG is elevated in the serum of individuals with DM compared with controls (Belfiore et al. 1972, Ferrara et al. 1979, Pitkanen et al. 1980). Studies of PMN from DM subjects suggest a normal lysosomal enzyme granule content (Sawant 1993) but diminished lysosomal enzyme release in response to challenge (Wierusz-Wysocka et al. 1987). Our data are consistent with the latter finding. BG activity was diminished among the subjects with DM in our study, yet there was a positive correlation between BG and pocket depth. A possible interpretation of this finding is that PMN are unable to migrate through the epithelial lining of the gingival sulcus, or are unable to degranulate once there.

Despite elevated circulating PMN and BG among DM subjects, a lower chemotactic activity (Mowat & Baum 1975, Delamaire et al. 1998) of PMN from subjects with DM could prevent an effective PMN response. Delamaire et al. (1998) reported increased adhesion molecule expression, and spontaneous activation of neutrophils among subjects with DM. That finding is consistent with elevated BG in the serum, and diminished BG in the gingival sulcus. Ineffective granule release is associated with decreased killing by PMN (McManus et al. 2001). In the gingival sulcus this deficiency would likely confer added risk for attachment loss.

Neutrophil dysfunction in DM, is possibly due to the direct or indirect

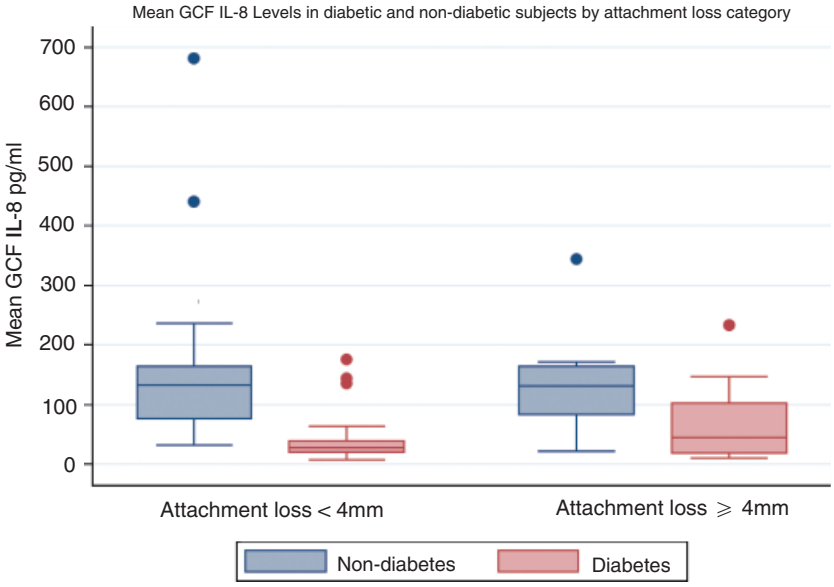

Fig. 3. Gingival crevicular fluid (GCF) interleukin-8 (IL-8) by attachment loss category in subjects with and without type 2 diabetes.

effects of hyperglycemia (Kjersem et al. 1988). Hyperglycemia has been shown to be associated with reduced PMN killing (Gallacher et al. 1995), though not always with reduced PMN chemotaxis (Donovan et al. 1987). We observed no statistically significant association between measures of hyperglycemia and GCF levels of either BG or IL-8. Alpagot et al. (2001) measured the PMN enzyme elastase in the GCF of type 1 and 2 DM, and also found no correlation with glycemic control.

A limitation of the present study is that we were not able to sample PMN from the subjects. Hence, we are not able to determine whether absolute numbers of PMN were lower in subjects with DM. Insufficient or ineffective PMN in the gingival sulcus would have important ramifications for periodontitis pathogenesis. Li et al. (2004) have recently determined that a critical neutrophil concentration is needed in order to maintain stable bacteria populations and prevent host infection. Studies of ex vivo stimulation of PMN from the gingival sulcus of subjects with DM and quantitative assessment of sulcular PMN are needed to explore whether lower BG in the GCF is indicative of a suboptimal critical neutrophil concentration.

Our finding of lower levels of BG is inconsistent with the findings of Oliver et al. (1993) who showed increased levels of GCF BG in patients with diabetes. The PD of the sampled site may explain these disparate findings. In our study, we sampled pre-determined anatomic locations within the periodontium rather than the most inflamed or diseased sites. Sampling sites with the most inflammation or increased PD will provide a bias towards increased BG levels as BG in the GCF are strongly associated with increased levels of inflammation and PD (Lamster et al. 1991). In our study, decreased levels of BG in patients with DM were observed regardless of PD, suggesting a systemic dysfunction of PMNs. The relationship of the diminished PMN response to IL-8 activity in DM subjects needs further study. Initial IL-8 levels are likely due to the presence of pathogenic subgingival microbiota (Jin et al. 2002). One deficiency in our own data is a lack of information regarding subgingival species and IL-8 production. This issue also needs to be addressed in future studies.

In summary, our data indicate a significant deficiency in neutrophil activity within the crevice of periodontitis patients with type 2 diabetes. A diminished neutrophil response may in part explain the increased susceptibility of type 2 diabetes patients to periodontal disease. Further, our data support the concept that the pathogenesis of chronic periodontitis and that of diabetes-related periodontitis are distinct. Future studies will be directed towards examining the relevance of these perturbations to progressive disease, and the use of specific drugs that may moderate the host response in periodontal disease occurring in the patients with diabetes.

Acknowledgements

This study was supported by a Columbia University Office of Clinical Trials Pilot Award (SE), and a training grant from The National Institute of Dental and Craniofacial Research award DE 00449 (SE).

References

Advani, A., Marshall, S. M. & Thomas, T. H. (2002) Impaired neutrophil actin assembly causes persistent CD11b expression and reduced primary granule exocytosis in Type II diabetes. *Diabetologia* **45**, 719–727.

Alpagot, T., Silverman, S., Lundergan, W., Bell, C. & Chambers, D. W. (2001) Crevicular fluid elastase levels in relation to periodontitis and metabolic control of diabetes. *Journal of Periodontal Research* **36**, 169–174.

Belfiore, F., Lo Vecchio, L. & Napoli, E. (1972) Serum beta-glucuronidase activity in diabetic patients as related to vascular complications and degree of glucose metabolic disorder. *American Journal of Medical Science* **264**, 457–466.

Chung, R. M., Grbic, J. T. & Lamster, I. B. (1997) Interleukin-8 and beta-glucuronidase in gingival crevicular fluid. *Journal of Clinical Periodontology* **24**, 146–152.

Collison, K. S., Parhar, R. S., Saleh, S. S., Meyer, B. F., Kwaasi, A. A., Hammami, M. M., Schmidt, A. M., Stern, D. M. & Al-Mohanna, F. A. (2002) RAGE-mediated neutrophil dysfunction is evoked by advanced glycation end products (AGEs). *Journal of Leukocyte Biology* **71**, 433–444.

Cutler, C. W., Eke, P., Arnold, R. R. & Van Dyke, T. E. (1991) Defective neutrophil function in an insulin-dependent diabetes mellitus patients. A case report. *Journal of Periodontology* **62**, 394–401.

Delamaire, M., Maugendre, D., Moreno, M., Le Goff, M. C., Allannic, H. & Genetet, B. (1998) Impaired leucocyte functions in diabetic patients. *Journal of Leukocyte Biology* **63**, 203–208.

Donovan, R. M., Goldstein, E., Kim, Y., Lippert, W., Kailath, E., Aoki, T. T., Cheung, A. T., Miller, M. E. & Chang, D. P. (1987) A computer-assisted image-analysis system for analyzing polymorphonuclear leukocyte chemotaxis in patients with diabetes mellitus. *Journal of Vascular Surgery* **5**, 787–790.

Engebretson, S. P., Hey-Hadavi, J., Ehrhardt, F. J., Hsu, D., Celenti, R. S., Grbic, J. T. & Lamster, I. B. (2004) Gingival crevicular fluid levels of interleukin-1beta and glycemic control in patients with chronic periodontitis and type 2 diabetes. *Journal of Periodontology* **75**, 1203–1208.

Engebretson, S. P., Lamster, I. B., Herrera-Abreu, M., Celenti, R. S., Timms, J. M., Chaudhary, A. G., di Giovine, F. S. & Kornman, K. S. (1999) The influence of interleukin gene polymorphism on expression of interleukin-1beta and tumor necrosis factor-alpha in periodontal tissue and gingival crevicular fluid. *Journal of Periodontology* **70**, 567–573.

Esposito, K., Nappo, F., Marfella, R., Giugliano, G., Giugliano, F., Ciotola, M., Quagliaro, L., Ceriello, A. & Giugliano, D. (2002) Inflammatory cytokine concentrations are acutely increased by hyperglycemia in humans: role of oxidative stress. *Circulation* **106**, 2067–2072.

Ferrara, M., Mizrahy, O., Saposhnik, A. & Feinstein, G. (1979) Serum beta-glucuronidase activity during pregnancy in gestational diabetes. *Isreal Journal of Medical Science* **15**, 746–748.

Figueredo, C. M. & Gustafsson, A. (2000) Increased amounts of laminin in GCF from untreated patients with periodontitis. *Journal of Clinical Periodontology* **27**, 313–318.

Gainet, J., Dang, P. M., Chollet-Martin, S., Brion, M., Sixou, M., Hakim, J., Gougerot-Pocidalo, M. A. & Elbim, C. (1999) Neutrophil dysfunctions, IL-8, and soluble L-selectin plasma levels in rapidly progressive versus adult and localized juvenile periodontitis: variations according to disease severity and microbial flora. *Journal of Immunology* **163**, 5013–5019.

Gallacher, S. J., Thomson, G., Fraser, W. D., Fisher, B. M., Gemmell, C. G. & MacCuish, A. C. (1995) Neutrophil bactericidal function in diabetes mellitus: evidence for association with blood glucose control. *Diabetic Medicine* **12**, 916–920.

Gamonal, J., Acevedo, A., Bascones, A., Jorge, O. & Silva, A. (2000) Levels of interleukin-1 beta, -8, and -10 and RANTES in gingival crevicular fluid and cell populations in adult periodontitis patients and the effect of periodontal treatment. *Journal of Periodontology* **71**, 1535–1545.

Geerlings, S. E., Brouwer, E. C., Van Kessel, K. C., Gaastra, W., Stolk, R. P. & Hoepelman, A. I. (2000) Cytokine secretion is impaired in women with diabetes mellitus. *European Journal of Clinical Investigation* **30**, 995–1001.

Giannopoulou, C., Kamma, J. J. & Mombelli, A. (2003) Effect of inflammation, smoking and stress on gingival crevicular fluid cytokine level. *Journal of Clinical Periodontology* **30**, 145–153.

Grbic, J. T., Lamster, I. B., Fine, J. B., Lam, K. S., Celenti, R. S., Herrera-Abreu, M. & Singer, R. E. (1999) Changes in gingival crevicular fluid levels of immunoglobulin. A following therapy: association with attachment loss. *Journal of Periodontology* **70**, 1221–1227.

Harper, D. S., Lamster, I. B. & Celenti, R. (1989) Relationship of subgingival plaque flora to lysosomal and cytoplasmic enzyme activity in gingival crevicular fluid. *Journal of Clinical Periodontology* **16**, 164–169.

Herder, C., Haastert, B., Muller-Scholze, S., Koenig, W., Thorand, B., Holle, R., Wichmann, H. E., Scherbaum, W. A., Martin, S. & Kolb, H. (2005) Association of systemic chemokine concentrations with impaired glucose tolerance and type 2 diabetes: results from the cooperative health research in the region of augsburg survey S4 (KORA S4). *Diabetes* **54**, S11–S17.

Iacono, V. J., Singh, S., Golub, L. M., Ramamurthy, N. S. & Kaslick, R. (1985) In vivo assay of crevicular leukocyte migration. Its development and potential applications. *Journal of Periodontology* **56**, 56–62.

Jin, L. J., Leung, W. K., Corbet, E. F. & Soder, B. (2002) Relationship of changes in interleukin-8 levels and granulocyte elastase activity in gingival crevicular fluid to subgingival periodontopathogens following nonsurgical periodontal therapy in subjects with chronic periodontitis. *Journal of Clinical Periodontology* **29**, 604–614.

Karima, M., Kantarci, A., Ohira, T., Hasturk, H., Jones, V. L., Nam, B. H., Malabanan, A., Trackman, P. C., Badwey, J. A. & Van Dyke, T. E. (2005) Enhanced superoxide release and elevated protein kinase C activity in neutrophils from diabetic patients: association with periodontitis. *Journal of Leukocyte Biology* **78**, 862–870.

Kjersem, H., Hilsted, J., Madsbad, S., Wandall, J. H., Johansen, K. S. & Borregaard, N. (1988) Polymorphonuclear leucocyte dysfunction during short term metabolic changes from normo- to hyperglycemia in type 1 (insulin dependent) diabetic patients. *Journal of Clinical Periodontology* **15**, 339–346.

Lamster, I. B., Holmes, L. G., Gross, K. B., Oshrain, R. L., Cohen, D. W., Rose, L. F., Peters, L. M. & Pope, M. R. (1995) The relationship of beta-glucuronidase activity in crevicular fluid to probing attachment loss in patients with adult periodontitis. Findings from a multicenter study. *Journal of Clinical Periodontology* **22**, 36–44.

Lamster, I. B. & Novak, M. J. (1992) Host mediators in gingival crevicular fluid: implications for the pathogenesis of periodontal disease. *Critical Reviews in Oral Biology and Medicine* **3**, 31–60.

Lamster, I. B., Oshrain, R. L., Celenti, R., Levine, K. & Fine, J. B. (1991) Correlation analysis for clinical and gingival crevicular fluid parameters at anatomically related gingival sites. *Journal of Clinical Periodontology* **18**, 272–277.

Lamster, I. B., Pullman, J. R., Celenti, R. S. & Grbic, J. T. (1996) The effect of tetracycline fiber therapy on beta-glucuronidase and interleukin-1 beta in crevicular fluid. *Technology and Health Care* **4**, 331–338.

Li, Y., Karlin, A., Loike, J. D. & Silverstein, S. C. (2004) Determination of the critical concentration of neutrophils required to block bacterial growth in tissues. *Journal of Experimental Medicine* **200**, 613–622.

Lin, X., Tan, W. C. & Candlish, J. K. (1995) Effect of glucose on the respiratory burst of circulating neutrophils from asthmatics. *Experimental and Molecular Pathology* **62**, 1–11.

Mathur, A., Michalowicz, B., Castillo, M. & Aeppli, D. (1996) Interleukin-1 alpha, interleukin-8 and interferon-alpha levels in gingival crevicular fluid. *Journal of Periodontal Research* **31**, 489–495.

McManus, L. M., Bloodworth, R. C., Prihoda, T. J., Blodgett, J. L. & Pinckard, R. N. (2001) Agonist-dependent failure of neutrophil function in diabetes correlates with extent of hyperglycemia. *Journal of Leukocyte Biology* **70**, 395–404.

Mowat, A. & Baum, J. (1975) Chemotaxis of polymorphonuclear leukocytes from patients with diabetes mellitus. *Endocrinology* **96**, 651–661.

Muchova, J., Liptakova, A., Orszaghova, Z., Garaiova, I., Tison, P., Carsky, J. & Durackova, Z. (1999) Antioxidant systems in polymorphonuclear leucocytes of type 2 diabetes mellitus. *Diabetic Medicine* **16**, 74–78.

Noack, B., Jachmann, I., Roscher, S., Sieber, L., Kopprasch, S., Luck, C., Hanefeld, M. & Hoffmann, T. (2000) Metabolic diseases and their possible link to risk indicators of periodontitis. *Journal of Periodontology* **71**, 898–903.

Noritake, M., Katsura, Y., Shinomiya, N., Kanatani, M., Uwabe, Y., Nagata, N. & Tsuru, S. (1992) Intracellular hydrogen peroxide production by peripheral phagocytes from diabetic patients. Dissociation between polymorphonuclear leucocytes and monocytes. *Clinical and Experimental Immunology* **88**, 269–274.

Oliver, R. C. & Tervonen, T. (1993) Periodontitis and tooth loss: comparing diabetics with the general population. *Journal of American Dental Association* **124**, 71–76.

Oliver, R. C., Tervonen, T., Flynn, D. G. & Keenan, K. M. (1993) Enzyme activity in crevicular fluid in relation to metabolic control of diabetes and other periodontal risk factors. *Journal of Periodontology* **64**, 358–362.

Ozmeric, N., Bal, B., Balos, K., Berker, E. & Bulut, S. (1998) The correlation of gingival crevicular fluid interleukin-8 levels and periodontal status in localized juvenile periodontitis. *Journal of Periodontology* **69**, 1299–1304.

Payne, J. B., Reinhardt, R. A., Masada, M. P., DuBois, L. M. & Allison, A. C. (1993) Gingival crevicular fluid IL-8: correlation with local IL-1 beta levels and patient estrogen status. *Journal of Periodontal Research* **28**, 451–453.

Pitkanen, E., Kyllastinen, M., Koivula, T. & Hormila, P. (1980) Beta-N-acetylglucosaminidase and beta-glucuronidase activities in insulin-dependent diabetic subjects with retinopathy. *Diabetologia* **18**, 275–278.

Salvi, G. E., Beck, J. D. & Offenbacher, S. (1998) PGE2, IL-1 beta, and TNF-alpha responses in diabetics as modifiers of periodontal disease expression. *Annals of Periodontology* **3**, 40–50.

Sawant, J. M. (1993) Biochemical changes in polymorphonuclear leucocytes in diabetic

patients. *Journal of Postgraduate Medicine* **39**, 183–186.

Schmidt, A. M., Hori, O., Brett, J., Yan, S. D., Wautier, J. L. & Stern, D. (1994) Cellular receptors for advanced glycation end products. Implications for induction of oxidant stress and cellular dysfunction in the pathogenesis of vascular lesions. *Arteriosclerosis and Thrombosis* **14**, 1521–1528.

Schmidt, A. M., Weidman, E., Lalla, E., Yan, S. D., Hori, O., Cao, R., Brett, J. G. & Lamster, I. B. (1996) Advanced glycation endproducts (AGEs) induce oxidant stress in the gingiva: a potential mechanism underlying accelerated periodontal disease associated with diabetes. *Journal of Periodontal Research* **31**, 508–515.

Soskolne, W. A. & Klinger, A. (2001) The relationship between periodontal diseases and diabetes: an overview. *Annals of Periodontology* **6**, 91–98.

Srinivasan, S., Yeh, M., Danziger, E. C., Hatley, M. E., Riggan, A. E., Leitinger, N., Berliner, J. A. & Hedrick, C. C. (2003) Glucose regulates monocyte adhesion through endothelial production of interleukin-8. *Circulation Research* **92**, 371–377.

Taylor, G. W., Burt, B. A., Becker, M. P., Genco, R. J., Shlossman, M., Knowler, W. C. & Pettitt, D. J. (1996) Severe periodontitis and risk for poor glycemic control in patients with non-insulin-dependent diabetes mellitus. *Journal of Periodontology* **67**, 1085–1093.

Tervonen, T. & Knuuttila, M. (1986) Relation of diabetes control to periodontal pocketing and alveolar bone level. *Oral Surgery Oral Medicine and Oral Pathology* **61**, 346–349.

Tonetti, M. S., Imboden, M. A., Gerber, L., Lang, N. P., Laissue, J. & Mueller, C. (1994) Localized expression of mRNA for phagocyte-specific chemotactic cytokines in human periodontal infections. *Infections and Immunity* **62**, 4005–4014.

Tsai, C., Hayes, C. & Taylor, G. W. (2002) Glycemic control of type 2 diabetes and severe periodontal disease in the US adult population. *Community Dentistry and Oral Epidemiology* **30**, 182–192.

Tsai, C. C., Ho, Y. P. & Chen, C. C. (1995) Levels of interleukin-1 beta and interleukin-8 in gingival crevicular fluids in adult periodontitis. *Journal of Periodontology* **66**, 852–859.

Wierusz-Wysocka, B., Wysocki, H., Siekierka, H., Wykretowicz, A., Szczepanik, A. & Klimas, R. (1987) Evidence of polymorphonuclear neutrophils (PMN) activation in patients with insulin-dependent diabetes mellitus. *Diabetes Research and Clinical Practice* **4**, 27–35.

Zozulinska, D., Majchrzak, A., Sobieska, M., Wiktorowicz, K. & Wierusz-Wysocka, B. (1999) Serum interleukin-8 level is increased in diabetic patients. *Diabetologia* **42**, 117–118.

Address:
Steven P. Engebretson
School of Dental Medicine
Stony Brook University
104 Rockland Hall
Stony Brook
New York 11794
USA
E-mail: sengebretson@notes.cc.sunysb.edu