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Gingival crevicular fluid interleukin-1β, prostaglandin E_2 and periodontal status in a community population

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Abstract

Aim: Interleukin-1 β (IL-1β) and prostaglandin E_2 (PGE_2) are key inflammatory mediators involved in periodontal disease. The purposes of this molecular cross-sectional epidemiological study were to investigate relationships in a community sample between mean concentrations of IL-1β and PGE_2 in gingival crevicular fluid (GCF) and (1) clinical periodontal signs and (2) risk factors of host inflammatory response and/or periodontal disease.

Material and Methods: The sample comprised 6277 community-dwelling adults aged 52–74 years enrolled in the Atherosclerosis Risk in Communities (ARIC) study. IL-1β and PGE_2 concentrations were measured using enzyme-linked immunosorbent assay. Person-level summary variables were computed for maximum pocket depth (MaxPD), maximum clinical attachment level (MaxCAL) and presence/absence of bleeding on probing (BOP). Mean GCF IL-1β and PGE_2 concentrations were dependent variables in multiple linear regression models with periodontal measures and covariates as explanatory variables.

Results: Both GCF IL-1β and PGE_2 were positively related to MaxPD and BOP in multiple regression models ($p<0.01$). Increased levels of IL-1β and PGE_2 were associated with body mass index $\geqslant 30\,kg/m^2$.

Conclusion: Higher levels of GCF IL-1β and PGE_2 were significantly associated with clinical signs of periodontal disease and independently related to patient-based anthropomorphic measures, behaviours and exposures in community-dwelling adults.

Key words: BMI; BOP; CAL; community; diabetes; GCF; IL-1β; PD; PGE_2; smoke

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Conflict of interest and source of funding statement

The authors declare that they have no conflict of interest.

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The presence and level of inflammatory products in gingival crevicular fluid (GCF) may be of diagnostic value in evaluating both periodontal disease status and the outcome of periodontal therapy (Alexander et al. 1996, Champagne et al. 2003). Inflammatory mediators interleukin-1 β (IL-1β) and prostaglandin E2 (PGE_2) are known to play a central role in host response as mediators of local tissue destruction and bone resorption (Offenbacher 1996, Roberts et al. 2004). Although clinical investigations have identified these inflammatory mediators within GCF as being elevated in periodontitis patients (Offenbacher et al. 1986, Masada et al. 1990, Alexander et al. 1996, Cavanaugh et al. 1998, Kido et al. 1999, McGuire & Nunn 1999), as increasing during periods of disease progression and as decreasing in response to therapy (Engebretson et al. 2002, Champagne et al. 2003, Gorska et al. 2003), little has been done to associate them with clinical signs of periodontal disease in community samples. Moreover, the levels of GCF inflammatory mediators are no doubt driven by a variety of genetic determinants and environmental

exposures in different populations. Ideally, the contribution of these factors should be considered when studying the association between clinical signs of periodontal disease and inflammatory mediators in GCF. The genotype/phenotype associations of the IL-1 gene cluster have been examined in some studies (Engebretson et al. 1999, Cutler et al. 2000, Shirodaria et al. 2000); however, no data regarding PGE_2 synthase or cyclooxygenase-2 (COX-2) polymorphisms are available. Further, few studies have adequately considered the potential impact of various patient-based risk factors [e.g., body mass index (BMI), diabetes, and hypertension] on the levels of these inflammatory mediators within GCF.

Recent literature continues to suggest that susceptibility to periodontitis varies greatly between individuals who harbour similar pathogenic microflora (Mølvig et al. 1988, Page & Beck 1997, Salvi et al. 1998). To date, the bulk of evidence points to the host response to bacterial challenge as a major determinant of susceptibility to periodontal disease and disease severity (Champagne et al. 2003). For instance, subgroups with certain systemic conditions, such as diabetes, may possess a monocytic hypersecretory trait (MO^+) that predisposes individuals to an exaggerated inflammatory response to periodontal pathogens (Salvi et al. 1998). Furthermore, the fundamental cellular and molecular mechanisms underlying relationships between various established risk factors for periodontal disease (e.g., smoking, diabetes, and BMI) and periodontal disease are not clear. Patient-based exposures or characteristics might act as risk factors for periodontal disease by altering the innate inflammatory response to an equivalent bacterial burden.

The purposes of the present study were to use the Atherosclerosis Risk in Communities (ARIC) study cohort for the following: (1) to investigate the association between clinical signs of periodontal disease and levels of GCF IL-1β and PGE_2 [We hypothesize that pocket depth (PD) and bleeding on probing (BOP) are positively associated with levels of GCF IL-1β and PGE_2 more strongly than clinical attachment level (CAL)] in a community-dwelling population; (2) to evaluate these associations after adjusting for covariates associated with host inflammatory burden and/or periodontal disease; and (3) to assess the effect of covariates on levels of GCF IL-1β and PGE_2. Although the subject level inflammatory response genes are likely determinants of the GCF inflammatory response, these data are not included in the present analyses.

Material and Methods

Study participants

Data used in this study were obtained from ARIC, a prospective study of 15,792 community-dwelling adults aged 45–64 conducted in four US communities: Forsyth County, North Carolina (NC); Jackson, Mississippi (MS); Minneapolis, Minnesota (MN); and Washington County, Maryland (MD). Participants were re-examined every 3 years following recruitment in 1987–1989. One major goal of ARIC was to investigate the aetiology and natural history of cardiovascular disease. In 1996–1998, the Dental ARIC (DARIC) was added as an ancillary cross-sectional study during the fourth ARIC visit when 6277 ARIC dentate participants aged 52–74 received an oral examination. Participants enrolled in the study provided informed consent to the protocol that was reviewed and approved by the Institutional Review Board on Research Involving Human Subjects at the University of North Carolina's School of Dentistry. The present study was based on information from subjects who had complete data from the clinical periodontal assessments and GCF samples ($n = 5809$ for IL-1β, $n = 6134$ for PGE_2).

Data collection

GCF was collected using Harco Periopaper (Tustin, CA, USA) before any probing measurements and was sampled from up to 16 periodontal pockets for each participant from the mesiobuccal and distobuccal sites of the two most posterior teeth present in each quadrant, excluding third molars. Briefly, a site was first isolated with cotton rolls and air dried, and the periopaper strip was placed gently into the crevice and left in place until the strip was visibly dampened. The fluid volume of the dampened strip was determined with a calibrated Harco Periotron. This volume was then used to compute the final mediator concentration. IL-1β and PGE_2 concentrations were measured independently on the same GCF strip, using enzyme-linked immunosorbent assays (ELISA, Caymen Chemical, Ann Arbor, MI, USA). For purposes of this study, IL-1β and PGE_2 concentrations were expressed as the average of all sampled sites to create person-level variables (i.e., mean IL-1β and mean PGE_2).

Periodontal clinical measurements were collected by calibrated examiners at six sites of all teeth for each subject. Analyses in this study used clinical measurements at the sites where GCF samples were collected. Summary variables were computed for each subject for maximum pocket depth (MaxPD), maximum clinical attachment level (MaxCAL) and presence or absence of BOP at one or more sites. In other words, for each person, MaxPD and MaxCAL were taken as the maximum value from all sites where GCF samples were collected. A subject was considered to have BOP if any of the GCF sampling sites had bleeding after probing. As sites were pre-selected and were not random representations of the subject's periodontal status, the scoring of periodontal severity was limited to just the site sampling data that included GCF mediators and clinical measures. Maximum values were chosen because preliminary analyses showed similar results between maximum and mean values of clinical measurements, and maximum values represent the most severe diseases of clinical interest.

A large number of socio-demographic and health status measures were available from standardized interviews designed by the parent ARIC protocol. Based on the literature, the following covariates were selected to be included in the multiple regression analyses: age at visit 4, BMI, total cholesterol value, personal oral hygiene/dental services (i.e., ''how often brushed teeth yesterday'', ''how often used floss last week'', ''last time saw dentist'', ''when visits dentist''), anti-inflammatory medication history (i.e., ''currently taking aspirin'', ''currently taking another non-steroidal anti-inflammatory drugs (NSAIDs)''), sex, education, study center/race, hypertension, reported or detected cardiovascular disease, smoking and diabetes history. Study centre/race was designed to control for the regional, ethnic and examiner differences in the ARIC cohort. Smoking history was categorized as five levels: current heavy, current light, former heavy, former light and never, with light smokers reporting 0–20 pack-years of smoking and heavy smokers reporting $\geqslant$20 pack-years of smoking.

Statistical analyses

The analysis unit was the person. Mean GCF IL-1β and GCF PGE_2 concentrations were the continuous dependent variables in the analyses, with three periodontal measures as explanatory variables of main interest. MaxPD and MaxCAL were categorized into three levels (i.e., 0–2, 3 and 4+ mm) to provide nominal indicators of periodontal destruction. BOP was used as a dichotomous variable (i.e., bleeding *versus* no bleeding).

Data were tabulated and bivariate statistical analyses between main explanatory variables and additional covariates with GCF IL-1β and GCF PGE2 were performed by type III F tests from analysis of variance. Those covariates and clinical signs of periodontal disease were further evaluated in multiple linear regression models. Two linear models were created for mean levels of GCF IL-1β and GCF PGE_2, respectively. In initial models, we first considered the effects of the three main exposure variables (MaxPD, MaxCAL and BOP) simultaneously. Next, we evaluated three two-way interactions among the main exposure variables (MaxPD MaxCAL, MaxPD × BOP and MaxCAL × BOP). Finally, we added all of the covariates into the resulting model and used a backward elimination strategy to formalize the final models. During backward elimination, the main exposure variables were retained in the model irrespective of statistical significance. Beginning with the full model, potential confounders with the largest *p*-values were removed if they did not lead to a significant change (i.e., at least 20%) in the estimates of the main exposures with the outcome. We chose 20% change because chance associations may arise over a large number of covariates in a big sample. Adjusted least-squares means were computed for each variable that remained in the final models. *p*-values for differences of the least-squares means with Tukey's adjustment for multiple comparisons were also evaluated. Statistical significance was set at 0.05. SAS Version 9.1 (Cary, NC, USA) was used for all analyses.

Results

A total of 5809 participants had complete information on GCF IL-1β and clinical periodontal assessments, while a total of 6134 participants had complete information on GCF PGE_2 and clinical periodontal assessments. Basic characteristics of the study population were described in Table 1.

Table 1. Subject characteristics of study population for two outcomes

Variables	Level	GCF IL-1β *n* (%)	GCF PGE2 *n* (%)
All		5809 (100.0)*	6134 (100.0)†
Maximum pocket depth	4+ mm	1345 (23.2)	1423 (23.2)
	3 mm	2422 (41.7)	2577 (42.0)
	0–2 mm	2042 (35.1)	2134 (34.8)
Maximum clinical attachment level	4+ mm	1382 (23.8)	1453 (23.7)
	3 mm	1543 (26.6)	1623 (26.5)
	0–2 mm	2884 (49.6)	3058 (49.8)
Bleeding on probing	Yes	3364 (57.9)	3578 (58.3)
	No	2445 (42.1)	2556 (41.7)
Age at visit in years (mean ± SD)		62.3 ± 5.6	62.4 ± 5.6
Body mass index	30+ kg/m^2	1896 (32.6)	2000 (32.6)
	25 − <30 kg/m^2	2403 (41.4)	2535 (41.3)
	13 − <25 kg/m^2	1503 (25.9)	1592 (26.0)
Total cholesterol value in mg/dl (mean ± SD)		201.1 ± 36.3	201.1 ± 36.1
''How often brushed teeth yesterday''	3+ times	743 (12.8)	781 (12.7)
	2 times	3311 (57.0)	3499 (57.0)
	0–1 time	1730 (29.8)	1829 (29.8)
''How often used floss last week''	3+ times	2688 (46.3)	2843 (46.3)
	1–2 times	1051 (18.1)	1103 (18.0)
	0 time	2044 (35.2)	2162 (35.2)
''Last time saw dentist''	2+ years	785 (13.5)	821 (13.4)
	05 − <2 years	1358 (23.4)	1429 (23.3)
	<0.5 year	3632 (62.5)	3850 (62.8)
''When visits dentist''	Not regular	1527 (26.3)	1598 (26.1)
	Regular	4255 (73.2)	4509 (73.5)
Currently taking Aspirin	Yes	1673 (28.8)	1769 (28.8)
	No	4122 (71.0)	4350 (70.9)
Currently taking another NSAID	Yes	750 (12.9)	789 (12.9)
	No	5046 (86.9)	5332 (86.9)
Sex	Male	2675 (46.0)	2827 (46.1)
	Female	3134 (54.0)	3307 (53.9)
Education	Advanced	2571 (44.3)	2698 (44.0)
	Intermediate	2480 (42.7)	2624 (42.8)
	Basic	753 (13.0)	806 (13.1)
Study center/race	MN, white	1988 (34.2)	2050 (33.4)
	MD, white	1263 (21.7)	1469 (23.9)
	NC, black	117 (2.0)	119 (1.9)
	NC, white	1423 (24.5)	1465 (23.9)
	Jackson, black	977 (16.8)	989 (16.1)
Hypertension	Yes	2780 (47.9)	2938 (47.9)
	No	3011 (51.8)	3178 (51.8)
Cardiovascular disease history	Yes	543 (9.4)	568 (9.3)
	No	5266 (90.6)	5566 (90.7)
Smoking history	Current heavy	553 (9.5)	567 (9.2)
	Current light	147 (2.5)	148 (2.4)
	Former heavy	956 (16.5)	1015 (16.5)
	Former light	1264 (21.8)	1344 (21.9)
	Never	2889 (49.7)	3060 (49.9)
Diabetes history	Yes	975 (16.8)	1030 (16.8)
	No	4810 (82.8)	5079 (82.8)

*Subjects who had complete information on GCF IL-1β and clinical periodontal assessments.
†Subjects who had complete information on GCF PGE_2 and clinical periodontal assessments.

Level of mean IL-1β in GCF (ng/ml)

Among 5809 participants who had complete information on GCF IL-1β and clinical periodontal assessments, the average level of mean IL-1β in GCF was 136.8 ± 1.4 (mean ± SE) ng/ml. In bivariate analyses, statistically significant associations were observed between all three clinical periodontal measures and mean GCF IL-1β concentration.

In multiple linear regression analyses, MaxPD and BOP persisted as independent,

Table 2. Multiple linear regression model describing relationships among pocket depth, clinical attachment level, and bleeding on probing with mean GCF IL-1β ($n = 5809$)

Independent variables	p-value*	Level	Adjusted mean IL-1β (ng/ml)†
Average			136.8 (1.4)
Maximum pocket depth	<0.01	4+ mm	155.0 (4.2)
		3 mm	147.3 (3.5)
		0–2 mm‡	130.0 (3.9)
Maximum clinical attachment level	0.32	4+ mm	145.8 (3.9)
		3 mm	141.0 (3.8)
		0–2 mm‡	145.4 (3.7)
Bleeding on probing	<0.01	Yes	159.2 (3.2)
		No‡	129.0 (3.5)
Body mass index	<0.01	30+ kg/m^2	151.1 (3.5)
		25 – <30 kg/m^2	142.4 (3.4)
		13 – <25 kg/m^2‡	138.7 (3.8)
''How often brushed teeth yesterday''	<0.01	3+ times	136.3 (4.5)
		2 times	143.0 (3.1)
		0–1 time‡	153.0 (3.5)
''When visits dentist''	0.04	Not regular	147.7 (3.6)
		Regular‡	140.5 (3.3)
Study center/race	<0.01	MN, white	159.9 (3.4)
		MD, white	146.4 (3.8)
		NC, black	155.6 (9.2)
		NC, white	162.0 (3.6)
		Jackson, black‡	96.6 (3.9)
Smoking history	<0.01	Current heavy	163.3 (4.7)
		Current light	154.7 (8.4)
		Former heavy	134.0 (4.0)
		Former light	134.1 (3.6)
		Never‡	134.4 (3.0)
Sex	0.04	Male	141.2 (3.3)
		Female‡	147.0 (3.3)
Diabetes history	<0.01	Yes	151.3 (4.0)
		No‡	136.9 (2.9)

*From type III F tests for analysis of variance.
†Adjusted means after controlling for other covariates in the model, represented as mean ± SE
‡Reference group.

statistically significant explanatory variables ($p<0.01$) for mean IL-1β concentration after controlling for BMI, ''how often brushed teeth yesterday'', ''when visits dentist'', study centre/race, smoking history, sex and diabetes history (Table 2). Mean IL-1β concentration (mean ± SE) was significantly higher ($p<0.01$) among individuals with higher MaxPD scores, increasing by 19.2% (4+ mm) as compared with individuals with lowest MaxPD scores. GCF IL-1β was also significantly 23.4% greater among people who had BOP than people who had no BOP ($p<0.01$). Neither MaxCAL nor any of the three tested interaction terms was significant in the final-adjusted model.

Evaluation of the covariates included in the multiple regression models indicated that people with BMI of 30+ kg/m^2 had a significantly higher mean IL-1β level than those with BMI of 13 <25 kg/m^2. In addition, mean IL-1β level was significantly lower among people who brushed their teeth at least twice a day before the examination than those brushing fewer than twice. People who visited a dentist on a regular basis also had a significantly lower mean IL-1β concentration than people who did not. Participants from MN, MD and NC had significantly higher mean IL-1β than those from Jackson County, MS, all of whom were blacks. Compared with never smokers, only current heavy smokers showed significantly higher mean IL-1β ($p<0.0001$, with Tukey's adjustment for multiple comparisons). Finally, mean IL-1β concentration was slightly higher among females, but 10.5% higher among individuals with diabetes history ($p<0.01$).

Level of mean PGE_2 in GCF (ng/ml)

Among 6134 participants who had complete information on GCF PGE_2 and clinical periodontal assessments, the average level of mean PGE_2 in GCF was 214.5 ± 1.8 (mean ± SE) ng/ml. Bivariate analyses revealed significant associations among all three clinical periodontal measures and mean GCF PGE_2 concentration.

Multiple linear regression analyses demonstrated that MaxPD and BOP remained as independent, significant explanatory variables for mean PGE_2, with the adjustment for BMI, ''last time saw dentist'', currently taking aspirin, study centre/race and smoking history (Table 3). Similar to mean IL-1β, a higher level of mean PGE_2 was seen among individuals with higher MaxPD scores or BOP. However, with regard to MaxCAL, people with 4+ mm MaxCAL had a significantly lower level of mean PGE_2 than those with 0–2 mm MaxCAL, the intermediate group (3 mm MaxCAL) did not show any significant difference when compared with the 0–2 mm group. There was an interaction between MaxCAL and BOP for mean GCF PGE_2, although insignificant ($p = 0.06$) (the interaction was left in the model as main effect variables). The effect of BOP on mean GCF PGE_2 level varies across different levels of MaxCAL, i.e., the effect of BOP on mean PGE_2 was modified by CAL. With the group of 0–2 mm MaxCAL and no BOP as a reference, linear regression resulted in least-square means (mean ± SE) of 180.6 ± 5.8, 172.5 ± 7.3, 218.1 ± 5.5, 195.1 ± 6.6 and 230.4 ± 5.4 ng/ml for groups with 4+ mm MaxCAL and bleeding, 4+ mm MaxCAL and no bleeding, 3 mm and bleeding, 3 mm and no bleeding, 0–2 mm and bleeding, respectively. Further analysis revealed that, for the reference attachment level group (0–2 mm) and the 3 mm attachment level group, BOP was significantly associated with a greater increase in mean PGE_2 levels compared with no bleeding ($p<0.0001$ for 0–2 mm group, $p = 0.0146$ for 3 mm group), whereas for people with attachment level of 4+ mm, BOP was not significantly associated with mean PGE_2 ($p = 0.9231$) (Fig. 1).

Among the covariates evaluated in the models, like mean GCF IL-1β, a significantly higher level of mean GCF PGE_2 was also observed among individuals with 30+ kg/m^2 BMI (*versus* 13 – <35 kg/m^2). Individuals from Jackson County, MS still had the significantly lowest mean PGE_2 compared with those from MN, MD and NC. Unlike mean GCF IL-1β, current heavy

Table 3. Multiple linear regression model describing relationships among pocket depth, clinical attachment level, and bleeding on probing with mean GCF PGE_2 ($n = 6134$)

Independent variables	*p*-value*	Level	Adjusted mean PGE_2 (ng/ml)†
Average			214.5 (1.8)
Maximum pocket depth	<0.01	4+ mm	209.2 (5.7)
		3 mm	201.0 (4.5)
		0–2 mm‡	188.1 (5.0)
Maximum clinical attachment level	<0.01	4+ mm	176.5 (5.2)
		3 mm	206.6 (4.9)
		0–2 mm‡	215.1 (4.8)
Bleeding on probing	<0.01	Yes	209.7 (4.1)
		No‡	189.1 (4.7)
Interaction: Maximum CAL ×	0.06	AL = 4+ mm; BOP = Yes	180.6 (5.8)
Bleeding on probing		AL = 3 mm; BOP = Yes	218.1 (5.5)
		AL = 0–2 mm and BOP = No‡	199.8 (5.5)
Body mass index	0.03	30+ kg/m^2	205.1 (4.6)
		25 – <30 kg/m^2	200.2 (4.4)
		13 – <25 kg/m^2‡	193.0 (4.9)
''Last time saw dentist''	0.01	2+ years	189.2 (5.8)
		0.5 – <2 years	207.4 (4.8)
		<0.5 year‡	201.6 (4.1)
Currently taking Aspirin	<0.01	Yes	194.1 (4.7)
		No‡	204.7 (3.9)
Study center/race	<0.01	MN, white	206.7 (4.3)
		MD, white	184.8 (4.7)
		NC, black	214.5 (12.5)
		NC, white	236.5 (4.7)
		Jackson, black‡	154.6 (5.1)
Smoking history	<0.01	Current heavy	185.4 (6.3)
		Current light	197.6 (11.4)
		Former heavy	198.9 (5.2)
		Former light	204.1 (4.7)
		Never‡	211.1 (3.8)

*From type III F tests for analysis of variance.
†Adjusted means after controlling for other covariates in the model, represented as mean ± SE
‡Reference group.

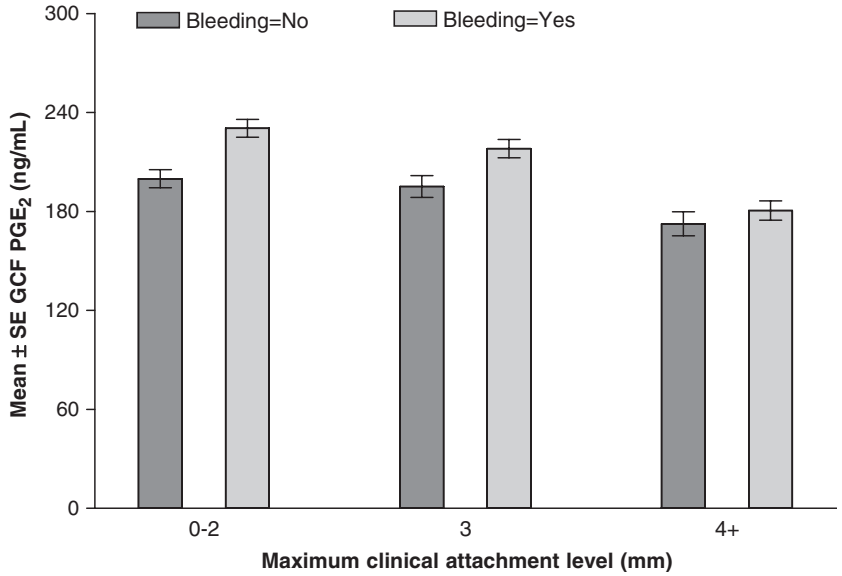

Fig. 1. Interaction between clinical attachment level and gingival bleeding on probing on level of mean GCF PGE_2 (ng/ml).

smokers demonstrated a significant lower level of GCF PGE_2 compared with never smokers. ''Last time saw dentist'' overall remained as a significant variable in the final model ($p = 0.01$), however, multiple comparisons with Tukey's adjustment revealed that, when compared with people who visited dentist in the past 6 months, those who did not visit dentist over 2 years only showed a slightly significant lower level of mean PGE_2 ($p = 0.067$), and people who did not visit dentist for 0.5 – <2 years did not show a significant difference ($p = 0.354$). Moreover, people reporting currently taking aspirin showed a significantly lower level of mean PGE_2 than those who did not.

Variables evaluated but not retained in the two final models due to non-significance include age, total cholesterol value, ''how often used floss last week'', ''currently taking another NSAIDs'', education, hypertension and reported or detected cardiovascular disease history.

Discussion

Inflammatory mediator levels within GCF have been considered as subclinical markers of the progression and severity of periodontitis, as well as indicators of response to treatment. In this study, both mean GCF IL-1β and GCF PGE_2 concentrations were higher among individuals with deeper PDs, or in the presence of BOP. These results confirm and extend previous findings of associations between clinical signs of periodontitis and GCF IL-1β and PGE_2 in clinical study patients to a community-dwelling population. In addition, we have shown that these clinical associations with each GCF inflammatory mediator were independent of one another and remained significant after controlling for other covariates.

Study results confirmed our hypothesis that CAL was not as strongly associated with increased levels of GCF IL-1β and PGE_2, as PD and BOP. This is likely because GCF inflammatory mediators are indicators of current inflammatory activity, whereas CAL represents the individual's total history of periodontal destruction, some of which may not have been active at the time of the examination. A prior publication on the same population showed that there is a great deal of variability in probing depths and BOP within each level of attachment loss (ALOSS) and that probing depth and BOP were more strongly associated with systemic markers of acute phase response and vascular stress (Beck & Offenbacher 2002). The earlier finding is consistent with probing depth and BOP being

strongly related to local measures of inflammation.

Specifically, 3 mm attachment level was not related to GCF PGE_2 and, 4+ mm attachment level was negatively associated with GCF PGE_2 compared with the reference group of CAL 0–2 mm ($p < 0.01$). This negative association was evident only for attachment level and not present for PDs or bleeding. The explanation for this is not readily apparent but could be partly due to the temporal changes in GCF PGE_2 levels associated with ALOSS. It has been proposed that GCF PGE_2 levels increase before the development of the tissue destruction and are highest during an ALOSS episode but then reduce (Offenbacher et al. 1986). It is hence speculated that PGE_2 levels increased and reached a peak value at the sites where periodontal tissue destruction and attachment loss were actually ongoing, and then decreased while attachment level deteriorated. In the current cross-sectional study, clinical examinations probably identified subjects with average CAL of 4+ mm, in whom periodontal sites currently were inactive, and hence had PGE_2 levels that were lower even than people who had sites with average CAL of 0–2 mm. On the contrary, individuals with 0–2 mm CAL had the highest mean PGE_2 levels and may be at risk for an oncoming ALOSS episode that might have been identified had these people been followed up. Although the cross-sectional design of the present study did not allow us to test this hypothesis, several publications have shown that, in people with periodontal disease, more shallow sites progress than deeper sites (Beck et al. 1990, Offenbacher et al. 2001). Moreover, the interaction between MaxCAL and BOP for mean GCF PGE_2 revealed that for the 0–2 and 3 mm MaxCAL groups bleeding was associated with increased GCF PGE_2, while for 4+ mm MaxCAL group bleeding was not significantly associated with GCF PGE_2. This finding further confirmed that GCF PGE_2 was not related to clinical measures such as BOP among individuals with 4+ mm MaxCAL.

Besides the main explanatory variables, our study had some interesting findings of covariates that we feel noteworthy of discussing. We will present them in the following paragraphs. Obesity has been considered as an important risk factor for periodontal disease (Saito et al. 2001, Alabdulkarim et al. 2005). Our study found individuals with BMI $\geqslant 30\,kg/m^2$ had significantly increased levels of GCF IL-1β and PGE_2. This result seems to be in agreement with a recent investigation that showed BMI positively correlated with GCF tumour necrosis factor α (TNF-α) in a group of young subjects with BMI $\geqslant 40\,kg/m^2$ (Lundin et al. 2004). Though BMI was found to be positively associated with levels of IL-1β and PGE_2 in whole blood supernatants and rectal mucosa (Bruunsgaard et al. 1999, Martinez et al. 1999), no studies as far as we know have looked at the association between BMI and IL-1β & PGE_2 in GCF. The elevated levels of GCF IL-1β and PGE_2 associated with higher BMI could be a result of local production of these inflammatory mediators in periodontal tissue induced by obesity. Another explanation is that GCF IL-1β and PGE_2 levels might be affected by obesity through a systemic effect of circulating adipokines, such as TNF-α, which were secreted by adipose tissue and contributed to the pro-inflammatory environment (Lyon et al. 2003). In response to adipokines enhanced by obesity, the production of IL-1β and PGE_2 in monocytes/macrophages may be also enhanced. Our findings suggest that GCF cytokines may act, in part, as intermediary explanatory variables in the link between obesity and risk for periodontal disease.

Among all types of smokers, only current heavy smokers showed significantly different results compared with never smokers. This suggests that both immediacy and intensity of smoking contribute to the effect of smoking on GCF cytokine profiles. Yet our data failed to reveal a consistent pattern of this effect. Heavy smokers at the time of examination had significantly higher mean GCF IL-1β (difference = 28.9 ± 4.7 ng/ml) and lower mean GCF PGE_2 than never smokers (difference = -25.7 ± 6.3 ng/ml). Our results are consistent with an earlier investigation that reported non-smokers had significantly higher GCF PGE_2 levels than smokers (Soder et al. 2002). However, our results differ from other studies (Payne et al. 1996, Rawlinson et al. 2003). Some other observations revealed no association between smoking status and IL-1β or PGE_2 in GCF (Heasman et al. 1998, Soder 1999, Bostrom et al. 2000, Giannopoulou et al. 2003). The reason for the negative effect of smoking on GCF PGE_2 observed in our study is not clear. However, the increased mean IL-1β among current heavy smokers might be due to their inhibited PGE_2 synthesis, since PGE_2 has been suggested to modulate IL-1β levels by an inhibitive feedback mechanism (Kunkel et al. 1986). Another noticeable observation in the present study was the insignificant differences in levels of GCF cytokine in former smokers compared with non-smokers. This seems to agree with a previous finding that suggested smoking cessation is beneficial to periodontal status while continuous smoking compromises the periodontal health (Bergstrom et al. 2000). The discrepancy between our study results and others might be due to variations in the study population, study design, GCF collection protocols and processing methodology. For instance, the study population in the present study is relatively mature (52–74 years old), their host inflammatory responses to chemical components in nicotine might be different from the younger population in other studies, as reflected by changes in levels of GCF pro-inflammatory mediators. Despite these differences, the inconsistent findings among literature suggest that the effect of smoking on GCF mediators is complex and further studies are needed to elucidate the underlying mechanisms.

There were four personal oral hygiene/dental services variables assessed in the present study in questionnaire formats: ''how often brushed teeth yesterday'', ''how often used floss last week'', ''last time saw dentist'', and ''when visits dentist''. In the final models, ''how often brushed teeth yesterday'' and ''when visits dentist'' remained as significant covariates for level of mean IL-1β, whereas ''last time saw dentist'' remained for mean PGE_2. However, as none of these variables has a consistent effect on mean IL-1β or mean PGE_2, these results should be interpreted with caution. First, personal oral hygiene and dental services variables may contain biases due to self-reporting. Second, the variables may not accurately reflect the immediacy or the cumulative history of hygiene or dental usage habits. For instance, ''how often brushed teeth yesterday'' may not be considered as accurate measurement reflecting history of personal oral hygiene information. The lower level of mean IL-1β among

individuals who brushed their teeth more often could possibly be an instant reduction from improved local gingival status due to recent brushing. The frequency of dental visits determined from ''When visits dentist'' is at borderline significance level ($p = 0.04$). Multiple comparisons revealed that the overall significance of ''last time saw dentist'' is probably due to the significant difference between the group with interval of ''2+ years'' and that with ''0.5 – <2 years'' (one possible explanation is that those with longest interval might have relatively healthier periodontal status), there is in fact no significant differences between these two groups and the reference group (with interval of ''<0.5 year''), respectively.

One may expect that the local microbial burden level of dental plaque could impact the local GCF levels of inflammatory mediators. To test the role of plaque in this relationship, we performed additional analyses with plaque included into the initial models. The results revealed a strong effect of plaque on IL-1β, i.e., mean levels of GCF IL-1β increased from 130.3, 143.8 to 156.5 ng/ml when plaque score changed from absence, covering <1/3 of the tooth surface to ⩾1/3 of the surface ($p < 0.01$). In this instance the effects of the oral hygiene/dental services variables became non-significant for IL-1β only, eliminating the frequency of dental visits (''when visits dentist'') but not ''how often brushed teeth yesterday''. However, a similar trend was not seen for the effect of plaque on mean levels of GCF PGE_2, and ''last time saw dentist'' remained significant in the model. On the other hand, including plaque into the analyses did not make significant impact on these model selections. For this reason we chose to include the oral hygiene/dental services variables rather than the direct plaque scores which are essentially interchangeable. Overall the findings indicate that the detected associations between the patient-based risk factors, the clinical measures, and local GCF levels of inflammatory mediators remain significant independent of local microbial burden.

There were additional covariates related to one but not both cytokines in the present study. For instance, diabetes was found to be only associated with increased level of GCF IL-1β but not PGE_2. Our finding of the elevated GCF IL-1β related to diabetes is consistent with previous reports in type 2 diabetes patients (Bulut et al. 2001, Engebretson et al. 2004) and type 1 diabetes patients (Salvi et al. 1998). Our study did not reveal the same association between diabetes and GCF PGE_2, this differs from previous findings that showed type 1 diabetes patients had significantly higher GCF levels of PGE_2 compared with non-diabetic controls with similar periodontal status (Salvi et al. 1998, Araya et al. 2003). Previous reports as to the elevated levels of GCF PGE_2 were only reported in type 1 diabetics, it could be possible that the present study is consisted of most type 2 diabetics who would show a different profile of GCF PGE_2. Whether or not the level of GCF PGE_2 differ among different types of diabetics is not clear and in ARIC the type of diabetes was unspecified, but likely type 2. Alternatively, those subjects in previous reports were selected as convenience samples of periodontal patients from clinical populations who might be expected to have a more aggressive form of periodontal disease than subjects from the community at large. Elevated GCF cytokines in diabetic patients could possibly be explained by an upregulated monocytic inflammatory secretion in response to lipopolysaccharides challenge induced by hyperglycemia (Schmidt et al. 1996, Southerland et al. 2005). Altered levels of GCF cytokines might suggest a mechanism underlying the association between diabetes and periodontal disease.

One major limitation of the present study is its cross-sectional design (i.e., both exposure and outcome are determined at the same time, it is often not possible to establish a temporal relationship between the exposure and outcome), thus the relationships reported here should not be interpreted as causal. However, this is the first study conducted in a large community-dwelling population to show the associations between clinical signs of periodontal disease and GCF mediators in the context of several established risk factors for periodontal disease (e.g., smoking, diabetes and BMI). Admittedly the analyses of a large sample size are subject to type 1 error (i.e., a true null hypothesis is incorrectly rejected). Also the magnitude of some of the differences is relatively small in the range of 10–20%. However, these effects are independent and can be additive. For example, the levels of GCF IL-1β in an obese, diabetic, smoker with poor oral hygiene can be elevated by approximately 72%. Thus, patient-based characteristics as well as local measures of periodontal status and microbial load make significant contributions to the local inflammatory response.

It is now widely accepted that the pathogenesis of periodontal diseases is more complex than the presence of virulent microorganisms. Individuals harbouring different sets of risk factors may respond differently to the same bacterial challenge through their altered host inflammatory response. To place our work in context, the present findings indicate that GCF IL-1β and PGE_2 may be attractive candidate molecules for monitoring ongoing periodontal inflammation in the community. Furthermore, it is our belief that periodontal disease represents an abnormal inflammatory response to the oral biofilm. Our findings indicate that patient-based characteristics including obesity, smoking and diabetes make subject-level contributions to the local-inflammatory response in a community setting. Longitudinal studies would be desirable to future explore the foundational role of cytokines in the associations between oral and systemic diseases.

In summary, there are three major new findings. First, previous findings of the role of GCF IL-1β and PGE_2 in cross-sectional assessments of periodontal disease status in smaller studies of clinic patients also appear to be applicable to a community-dwelling population. Second, 30+ kg/m^2 BMI is significantly associated with increased levels of GCF IL-1β and PGE_2. To our knowledge, this is the first report to demonstrate the effect of BMI on levels of GCF IL-1β and PGE_2. Third, individuals with current heavy smoking and diabetes history had altered levels of GCF mediators that distinguish them from other groups in the community.

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Clinical Relevance

Scientific rationale for the study: Knowledge regarding the association of GCF IL-1β & PGE_2 with clinical signs and patient-based risk factors in community setting is limited.
Principal findings: The previously observed associations between PD, BOP and GCF IL-1β & PGE_2 in clinical setting can be extended to a community sample. Obesity, smoking and diabetes all contribute to periodontal inflammation.
Practical implications: Local levels of GCF IL-1β & PGE_2 might be of diagnostic value for periodontal status in not only patients but also community samples. Furthermore individuals with obesity, current heavy smoking and diabetes history possess higher levels of GCF-IL-1β and/or PGE_2 in excess of the effect associated with more severe periodontal destruction.