ORIGINAL REPORT: EPIDEMIOLOGIC RESEARCH

Gingival Fluid Inflammatory Biomarkers and Hypertension in African Americans

A. Khocht¹, T. Rogers², M.N. Janal³, and M. Brown⁴

Abstract: Hypertension and periodontitis are highly prevalent among African Americans. This cross-sectional study investigated the relationships among inflammatory biomarkers in the gingival fluid, periodontitis, and blood pressure in these individuals. Twenty-one subjects who reported a diagnosis of hypertension and a comparison group of 26 nonhypertensive participants were enrolled. All were African Americans, non-cigarette smoking, and free from other systemic illness. Blood pressure and body mass index were assessed. A comprehensive periodontal examination was performed. Gingival fluid was collected from 3 healthy sites and 3 diseased sites when available. Samples were assessed for 8-isoprostane, interleukin 1 β , monocyte chemoattractant protein 1, tumor necrosis factor- α (TNF α), C-reactive protein (CRP), and matrix metalloproteinase 8. Regardless of hypertension status, diseased sites were associated with increased levels of these biomarkers. CRP and TNF α levels were also significantly higher in hypertensive than nonhypertensive

individuals without diseased sites. After adjusting for demographics and body mass index, periodontal attachment loss was higher among hypertensive than nonhypertensive subjects. TNF α and CRP levels and hypertension were in the same association pathway with attachment loss. Elevated blood pressure may increase the risk of periodontitis through a localized inflammatory mechanism.

Knowledge Transfer Statement:

The results of this study can be used by clinicians to better understand the etiology and pathogenesis of periodontitis in hypertensive individuals in general and African Americans in particular. The information could lead to better management of periodontal disease.

Keywords: cytokines, tumor necrosis factor- α , C-reactive protein, periodontium, periodontitis, vascular diseases

Introduction

Periodontitis is a common oral disease characterized by periodontal tissue destruction. It is well documented

that African Americans (AAs) have an increased risk for developing periodontitis as compared with Caucasians (Borrell et al. 2005) as well as other minority racial groups living in the United States (Borrell and Crawford 2008). The reasons for this greater susceptibility to periodontitis in AAs are not fully understood. Race may represent a range of unmeasured exposures (i.e., racial discrimination, segregation, environmental exposure, unequal opportunities for social mobility, access to quality of care) in US society that may act directly or indirectly on periodontal diseases and other health conditions (Borrell and Crawford 2008).

Hypertension (HTN) is a medical condition characterized by chronic elevation of arterial blood pressure. A diagnosis of HTN is made when a person's systolic blood pressure is consistently ≥ 140 mm Hg or when the diastolic blood pressure is consistently ≥ 90 mm Hg. HTN is associated with altered collagen metabolism (Marchesi et al. 2012), elevated systemic inflammation, and increased oxidative stress (Vaziri 2008; Virdis et al. 2014) and is a risk factor for several diseases,

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particularly heart failure and stroke. It is well documented that AAs have the highest rates of HTN of any other race or ethnic type (Stewart et al. 2006; Minor et al. 2008).

Recent epidemiologic reviews (Tsioufis et al. 2011; Leong and Ng 2014; Macedo Paizan and Vilela-Martin 2014) concluded that hypertensive individuals have increased rates of periodontitis. Animal studies showed that HTN reduces the quality of tooth-supporting bone (Bastos et al. 2010) and is associated with progressive alveolar bone loss even when the stimulus for periodontal disease induction is removed (de Medeiros Vanderlei et al. 2013). The exact mechanisms linking the 2 diseases are not known. Altered collagen metabolism, oxidative stress, and inflammation are potential links between periodontal disease and HTN. It is possible that the inflammation associated with HTN may exacerbate the gingival inflammatory response to periodontopathic bacteria, leading to periodontal tissue damage. If true, this might explain the increased prevalence of periodontitis in hypertensive AAs.

Plaque-related inflammatory periodontal disease is associated with elevated levels of several important inflammatory biomarkers in gingival fluid (Gupta 2013): interleukin 1 β (IL-1 β ; Baser et al. 2009; Sanders et al. 2009), 8-isoprostane (8-iso; Pradeep et al. 2013), monocyte chemoattractant protein 1 (MCP-1; Gupta et al. 2013), tumor necrosis factor α (TNF α ; Baser et al. 2009), C-reactive protein (CRP; Sibraa et al. 1991; Megson et al. 2010), and matrix metalloproteinase 8 (MMP-8; Passoja et al. 2008; Teles et al. 2010).

The objectives of this study were to compare the levels of 6 gingival fluid inflammatory markers known to be associated with periodontitis (8-iso, IL-1 β , MCP-1, TNF α , CRP, and MMP-8) between nonhypertensive and hypertensive subjects and to investigate the relationships among inflammatory biomarkers in the gingival fluid, periodontitis, and blood pressure in these individuals.

Materials and Methods

This cross-sectional study was conducted at the Temple University School of Dentistry (Philadelphia, PA) from January 2010 to December 2012. The study protocol and consent forms were approved by the Temple University Institutional Review Board. AAs within the city of Philadelphia were recruited via newspaper advertisements. The study included 2 subject groups: a hypertensive group and a nonhypertensive comparison group.

The inclusion criteria for hypertensive subjects were as follows: self-reported history of a diagnosis of HTN by a physician, blood pressure between 140/90 and 160/100 mm Hg, no evidence or history of other medical conditions (including diabetes and immune dysfunction), age 35 to 75 y, a minimum of 10 teeth present, no antibiotic treatment in the past 3 mo prior to entry in the study, no medications with anti-inflammatory/antioxidative stress properties, no cigarette smoking for at least 6 mo prior to enrolling in the study, alcohol consumption <3 drinks per day, and ability to communicate in English. Hypertensive individuals who were on >1 antihypertensive medication, who reported a diagnosis of HTN but whose blood pressure was controlled with treatment at <140/90, and those with periodontal diseases other than plaque-related gingivitis and chronic periodontitis were excluded. Inclusion/exclusion criteria for the nonhypertensive subjects were the same as for the hypertensive subjects except for absence of any indications of HTN. On the group level, the normotensive and hypertensive subjects were matched on sex, race, age, and socioeconomic status.

Subject Screening and Enrollment

Individuals who responded to advertisements that publicized the project were initially contacted by telephone to assess eligibility. A total of 100 subjects were invited for in-person evaluations; 65 fulfilled all entry criteria and were enrolled; and 55 completed

all study evaluations. Written informed consent was obtained from all subjects.

Medical History Interview, Body Mass Index, and Blood Pressure Determination

For all subjects, a comprehensive medical history interview was performed. Subjects were asked, "Have you ever been diagnosed with elevated blood pressure (hypertension) by a medical doctor?" Any medications were noted. Height and weight measurements were recorded and body mass index (BMI) calculated.

Laboratory personnel measured blood pressure levels from seated subjects in accordance with JNC 7 guidelines on 3 separate visits (approximately 1 wk apart) in a quiet temperature-controlled room (Chobanian et al. 2003) with an aneroid sphygmomanometer (model 11-675D; Omron) after 5 min of quiet rest. For each visit, blood pressure measurements were repeated until values disagreed by ≤ 4 mm Hg. The average of 3 blood pressure values was used in data analyses.

Clinical Assessment and Sample Collection Visit

After completion of the 3 blood pressure assessment visits, subjects were scheduled for a periodontal clinical assessment visit within 15 d. Prior to the clinical examination, all participants answered a questionnaire related to education, income, dental hygiene practice, dental care, and history of cigarette smoking.

All subjects received a comprehensive oral/periodontal evaluation, including probing measurements. A single experienced dental examiner (A.K.) performed all dental examinations. The examiner was blinded to the patients' medical history and blood pressure status. The examiner was calibrated and standardized in the use of the clinical evaluation measures employed in the study. The agreement of the intraexaminer probing measures (differences within 1 mm) was 98%. The examiner recorded the plaque index (PI) and the gingival index (GI) around all teeth present (Loe 1967). Third molars,

partially erupted teeth, surfaces with large restorations, and teeth with crowns were not scored. Caries and missing teeth were also recorded.

A conventional periodontal probe (Michigan-O probe with Williams markings; Hu-Friedy) was used for all probing measurements. Probing depth (PD) was recorded on 6 sites per tooth: mesiobuccal, buccal, distobuccal, mesiolingual, lingual, and distolingual. The position of the gingival margin to the cemento-enamel junction was recorded at the same 6 sites per tooth. Periodontal attachment level (AL) was calculated per the following formula: $AL = PD + \text{gingival margin}$. Interproximal measurements of PD and AL were used to determine periodontal disease status per the definition of the Centers for Disease Control and Prevention and the American Academy of Periodontology for mild, moderate, and severe periodontal disease (Eke et al. 2012). Mild periodontitis was defined as the presence of ≥ 2 interproximal sites with $AL \geq 3$ mm and ≥ 2 interproximal sites with $PD \geq 4$ mm or 1 site with $PD \geq 5$ mm. Moderate periodontitis was defined as the presence of ≥ 2 teeth with $AL \geq 4$ mm or ≥ 2 teeth with $PD \geq 5$ mm. Severe periodontitis was defined as the presence of ≥ 2 teeth with $AL \geq 6$ mm in any of the interproximal sites and ≥ 1 tooth with $PD \geq 5$ mm at any interproximal site.

Gingival Fluid Sampling and Analysis

Gingival crevicular fluid (GCF) was collected by filter paper strips (Oraflow Inc.). For the purposes of GCF sampling, nondiseased sites were defined as having $PD \leq 3$ mm, no bleeding on probing, and no loss of attachment and diseased sites as having $PD > 4$ mm with equivalent or greater attachment loss. In periodontitis subjects, 3 interproximal diseased sites and 3 interproximal nondiseased sites were selected. In nonperiodontitis subjects, 3 interproximal nondiseased sites were selected. All selected sites were chosen at random from teeth in separate quadrants.

The selected sites were isolated, the supragingival plaque removed, and the tooth surface air-dried prior to sample collection. The strip was gently inserted

into the orifice of the gingival crevice 1 to 2 mm and left for 30 s. Sample volume was assessed with Periotron 8000 (Oraflow Inc.). The 3 nondiseased samples collected from each subject were pooled in 1 microcentrifuge tube; the 3 diseased samples collected from each periodontitis subject were also pooled in a microcentrifuge tube. Samples visibly stained with blood were discarded. Following sample collection, the tubes were sealed, placed on ice, and subsequently frozen at -70°C until testing was done.

GCF samples were analyzed for the presence of 6 inflammatory biomarkers known to be associated with periodontitis: 8-iso, IL-1 β , MCP-1, TNF α , CRP, and MMP-8. At the time of analysis, the 3 strips in each vial were immersed in 1 mL of elution buffer composed of phosphate buffered saline containing 0.1% Triton 100 and 0.1% bovine serum albumin (Aboyoussef et al. 1998). The elution process was carried out overnight under refrigeration (Aboyoussef et al. 1998). A set of controls for each biomarker (in which a known concentration of each biomarker was added to the filter papers) was used to mimic the elution of the investigated biological factors from the filter paper strips to calculate the percentage recovery. The percentage recovery for each biomarker was determined to be at least 98%. The standard curves were generated in the same elution buffer, thus maintaining matrix integrity.

The levels of 8-iso, CRP, and MMP-8 were determined with standard enzyme-linked immunosorbent assays (ELISAs) in which matching capture and biotinylated detection antibodies were employed for sandwich ELISA analysis (8-iso: Cayman Chemical Company; CRP: EBioscience, Inc.; MMP-8: R&D Systems). The levels of MCP-1, TNF α , and IL-1 β were determined with the Luminex magnetic beads (EMD Millipore) coated with specific antibodies (Milliplex MAP Kit). Fluorescence was read with a Luminex 200 instrument (Millipore). Analytes were normalized to total protein concentration, which was determined by Bradford assay (with elution buffer as a baseline).

Statistical Analysis

A priori power analysis indicated that a minimum sample size of 17 subjects per HTN group is required for 80% power to detect a clinically meaningful difference of approximately 0.5 mm (0.5 SD) in periodontal ALs in a *t* test at alpha of 0.05.

Subjects were divided into 2 groups based on reported history of HTN. The significance of mean differences between groups for continuous measures of blood pressure and dental clinical parameters was determined with analysis of variance, while differences between percentages (eg, males, those with a history of cigarette smoking, and those with periodontitis) was determined with chi-square test. The distribution of TNF α GCF levels was bimodal and dichotomized to “high” and “low” for analysis. The distributions of all other biomarker levels were positively skewed and rank transformed to correct that skew prior to analysis.

Multiple linear regression analysis was used to determine the significance of differences in biomarker levels as a function of HTN group, PD, and their interaction and to test the hypothesis that HTN and GCF biomarkers are in the same association pathway with AL. Plausible confounders were selected a priori according to the existing literature and included as covariates in the regression models. First, we selected a limited number of potential covariates—those needed to test the hypothesis but not necessarily all with a potentially confounding influence—in deference to the limited sample. Then, we used a backward elimination process, removing variables with $P > 0.05$. A type 1 error rate of 5% was used.

Reporting of this study conforms to the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) guidelines (von Elm et al. 2007).

Results

Twenty-one subjects reported a history of HTN diagnosed by a physician: their systolic blood pressure ranged between 140 and 159 mm Hg, or their diastolic

Table 1.

Demographics, BP, and Periodontal Clinical Measures.

	Nonhypertensive (<i>n</i> = 26)	Hypertensive (<i>n</i> = 21)	<i>P</i> Value
Demographics and BP measures			
Age, y	52.3 (1.7)	52.0 (1.9)	NS
Male	30.8	23.8	NS
Smoking history	34.6	28.6	NS
Systolic BP	119.6 (1.6)	143.7 (1.8)	0.001
Diastolic BP	76.7 (1.0)	92.9 (1.1)	0.001
Mean arterial BP	91.0 (1.1)	109.8 (1.2)	0.001
Body mass index	29.0 (1.1)		0.001
Access to dental care	73	76	NS
College education	48	32	NS
Periodontal clinical measures			
Teeth number	25.0 (0.6)		NS
Plaque index		23.7 (0.6)	NS
Gingival index	0.87 (0.1)		NS
GCF volume, μ L		0.84 (0.1)	NS
BOP	0.71 (0)		NS
PD, mm		0.76 (0)	NS
AL, mm	0.61 (0.1)	0.75 (0.1)	NS
Periodontitis	61	62	NS
Mild	19	19	
Moderate	19	24	
Severe	23	19	

Data are presented as mean (SE) or percentage. *P* value gives the probability that the groups differ in either analysis of variance test or chi-square test for means or proportions, respectively.

AL, attachment level; BOP, bleeding on probing; BP, blood pressure; GCF, gingival crevicular fluid; NS, not significant ($P > 0.05$); PD, probing depth.

blood pressure ranged between 90 and 99 mm Hg. Thirty-four subjects reported no history of HTN; of these, 8 were excluded from further analysis, with blood pressure measures consistently approaching 140/90 mm Hg.

Table 1 summarizes the demographic and blood pressure data by HTN status. Age, sex, history of smoking, access to professional dental care, and education were comparable between the 2 groups.

As would be expected, the hypertensive subjects showed higher systolic and diastolic blood pressure, higher mean arterial pressure, and higher BMI than nonhypertensive subjects. BMI was positively correlated with both diastolic ($r = 0.5$, $P < 0.01$) and systolic ($r = 0.4$, $P < 0.01$) blood pressure. All subjects reported practicing oral hygiene at least once per day. Table 1 also summarizes the clinical periodontal measures by

HTN status. Univariate analyses showed statistically similar levels of PI, GI, GCF volume, bleeding on probing, PD, AL, and the proportion of subjects with periodontitis between those with and without a diagnosis of HTN.

While the HTN groups showed comparable levels of AL in univariate analyses, those analyses did not control for known risk factors for periodontitis, including age, sex, cigarette smoking,

Figure 1. Bar chart of attachment level (mm) adjusted for demographics, history of cigarette smoking, body mass index, and plaque index. The bars represent the mean, and the whiskers indicate the standard error of the mean (SEM). Regression analysis adjusting for the above factors showed almost twice as much AL in hypertensive (HTN) as non-HTN subjects. * $F(6, 40) = 4.74, P = 0.03$.

BMI, and oral hygiene. Multivariate analyses that adjusted for these factors continued to show statistically similar levels of PI, GI, GCF volume, bleeding on probing, and PD (data not shown) but almost twice as much AL in HTN as non-HTN subjects, $F(6, 40) = 4.74, P = 0.03$ (Fig. 1). Thus, despite comparable levels of plaque and gingival inflammation, hypertensive subjects had greater loss of periodontal attachment than nonhypertensive subjects.

Table 2 shows mean biomarker levels and $\text{TNF}\alpha$ rates as a function of HTN and PD. Regression analysis (transformed data) showed higher rates of $\text{TNF}\alpha$ and higher levels of all other biomarkers in subjects with deep pockets (all $P < 0.05$). In subjects with shallow pockets (ie, those without current evidence of disease), higher levels of CRP were found in the GCF of hypertensive than normotensive subjects ($P = 0.02$). Additional analyses that adjusted for periodontal status and BMI continued to show, in subjects with shallow pockets, higher levels of CRP, $F(3, 29) = 4.19, P = 0.04$, as well as higher rates of $\text{TNF}\alpha$, $\chi^2 = 8.79, P = 0.003$, in HTN than non-HTN individuals (Fig. 2).

Last, we used a series of regression models to test our hypothesis that HTN

and GCF inflammatory biomarkers are in the same association pathway with AL (Table 3). Model 1 shows, as above, no association between HTN and AL. Model 2, which adjusts for history of tobacco smoking, BMI and PI, shows a positive association between HTN and AL ($P < 0.05$) and a notably improved model fit ($R^2 = 0.28$). In model 3, $\text{TNF}\alpha$ (healthy site samples from all subjects, dichotomized as “high” and “low”) was positively associated with AL ($P < 0.05$) and nullified the significance of the associations of HTN and BMI with AL. In model 4, CRP ranks (healthy site samples from all subjects) were positively associated with AL ($P < 0.01$) and also nullified the significance of the association between HTN and AL.

Discussion

An association between HTN and periodontal disease has been reported (Inoue et al. 2005; Tsakos et al. 2010; Rivas-Tumanyan et al. 2013), but little attention has been paid to the inflammatory mechanisms shared by both diseases. This study attempted to investigate the effect of blood pressure on gingival inflammatory mediators and AL. To test the hypothesis that

inflammatory mediators may be elevated in the gingival fluid of hypertensive subjects, we investigated an array of inflammatory biomarkers known to be associated with periodontitis. We sampled healthy and, when available, diseased gingival sites. Furthermore, in our data analysis, we controlled for the possible influence of demographics, BMI, plaque levels, and periodontal status (Figueredo et al. 1999). After adjusting for confounders, particularly current evidence of plaque and obesity, hypertensive subjects showed greater AL than nonhypertensive individuals. Furthermore, 2 of the biomarkers investigated, CRP and $\text{TNF}\alpha$, were positively influenced by HTN status, and both were in the same association pathway for AL as HTN.

CRP is an acute-phase protein that is mainly produced by the liver in response to inflammatory cytokines produced by activated inflammatory and other cells. Its main function is the activation of the complement system that helps with the clearance of bacteria and necrotic cells. Levels of CRP are elevated in the serum (Cairo et al. 2008) and GCF of periodontitis patients (Sibraa et al. 1991; Tüter et al. 2007; Sanders et al. 2009). In hypertensive subjects, CRP serum levels are elevated and positively associated with endothelial dysfunction, atherosclerosis, and the development of end-organ damage (Hage 2014). The significantly higher levels of CRP in the gingival fluid of hypertensive individuals noted in this study is most likely of systemic origin (Megson et al. 2010) and reflects their increased serum CRP levels (Tüter et al. 2007). Megson et al. (2010) investigated the gene expression of CRP in gingival tissues and found no detectable levels of CRP mRNA.

$\text{TNF}\alpha$ is an important proinflammatory cytokine that is produced by activated macrophages and other inflammatory cells, including T cells. It also plays a role in the regulation of immune cells and osteoclasts involved in bone remodeling (Zhang et al. 2001). $\text{TNF}\alpha$ is elevated in the GCF of participants with gingival inflammation and periodontitis

Table 2.
Biomarker Levels by Hypertension and PD Status.

	Nonhypertensive	Hypertensive	P Value
Deep PD ^a	<i>n</i> = 5	<i>n</i> = 9	
8-iso	339.9 (42.4)	354.4 (31.64)	NS
IL-1 β	7.5 (1.9)	5.5 (1.4)	NS
MCP-1	3.7 (2.3)	6.9 (1.7)	NS
TNF α	3.4 (0.4)	3.9 (0.3)	
Subjects >2 pg, %	80	100	NS
CRP	3,440.4 (1,924.0)	3353.0 (1434.1)	NS
MMP-8	148,087.0 (58,423.0)	169057.0 (43546.0)	NS
Shallow PD ^b	<i>n</i> = 21	<i>n</i> = 12	
8-iso	188.6 (23.5)	260.9 (31.2)	NS
IL-1 β	2.42 (0.7)	1.3 (0.9)	NS
MCP-1	2.1 (0.3)	2.9 (0.4)	NS
TNF α	1.7 (0.3)	2.5 (0.3)	
Subjects >2 pg, %	43%	75%	NS
CRP	671.2 (370.8)	1916.8 (490.6)	0.02
MMP-8	71,874.6 (13,926.0)	86,245.3 (18,423.0)	NS

Data presented as mean (SE) for all biomarkers (pg) and as percentage of subjects with high TNF α (>2 pg). Regression analysis (transformed data) showed higher CRP levels in hypertensive than normotensive subjects with shallow PD, statistically similar levels for all other biomarkers between hypertension groups (all $P > 0.05$), higher TNF α rates and higher levels of all other biomarkers in subjects with deep pockets (all $P < 0.05$), and no significant interactions between hypertension and PD on TNF α rates and all other biomarker levels (all $P > 0.2$).

8-iso, 8-isoprostane; CRP, C-reactive protein; IL-1 β , interleukin 1 β ; MCP-1, monocyte chemoattractant protein 1; MMP-8, matrix metalloproteinase-8; NS, not significant ($P > 0.05$); PD, probing depth; TNF α , tumor necrosis factor α .

^aDeep PD: subjects with PD ≥ 4 mm (3 pooled healthy and 3 pooled diseased samples; total of both samples averaged within each subject, then averaged across subjects).

^bShallow PD: subjects with PD <4 mm (total of 3 pooled samples collected from healthy sites, then averaged across subjects).

(Baser et al. 2009; Ertugrul et al. 2013). TNF α has been positively associated with increased blood pressure in humans (Furumoto et al. 2002) and could be an independent risk factors for elevated blood pressure in apparently healthy subjects (Bautista et al. 2005). TNF α is involved in mediating AngII-induced effects on HTN and cardiac hypertrophy in experimental animals (Sriramula et al. 2008). CD8+ T cells are increased in hypertensive participants and are an important source of TNF α (Youn et al. 2013). The elevated levels of TNF α in the GCF of hypertensive subjects noted in this study may upregulate RANKL

production in the periodontal tissues and contribute to increased osteoclastogenesis and alveolar bone loss.

We used a series of regression analyses to investigate the associations among HTN, inflammatory biomarkers, and loss of periodontal attachment. Table 3 (model 3) shows that TNF α was positively associated with AL and nullified the significance of the association of AL with HTN and BMI. This suggests that the associations between AL and both HTN and BMI can be partially explained by TNF α . Table 3 (model 4) shows that CRP was also positively associated with AL

and nullified the significance of the association between AL with HTN. This suggests that the association between AL with HTN can also be partially explained by CRP. Unlike TNF α , CRP and BMI were independently associated with AL. Furthermore, regression analyses used to generate Figure 2, indicated that GCF CRP levels in nondiseased sites are primarily attributable to HTN, as neither BMI nor periodontitis showed a significant association with GCF CRP levels. These results are consistent with the proposed hypothesis that HTN is an independent risk for inflammation and loss of periodontal attachment in AAs.

Figure 2. Bar charts of tumor necrosis factor α (TNF α) and C-reactive protein (CRP) from gingival crevicular fluid sampled from subjects with shallow sites only. TNF α data (dichotomous) are presented as percentages of subjects with TNF α >2 pg. Regression analysis adjusting for periodontitis and body mass index showed higher rates of elevated TNF α in hypertensive (HTN) than non-HTN individuals. $\chi^2 = 8.79$, $P = 0.003$. CRP data (back-transformed) are presented as mean (SEM): the bars represent the mean, and the whiskers indicate the SEM. Regression analyses (rank-transformed data) that adjusted for periodontitis and body mass index showed higher CRP levels in the gingival crevicular fluid of HTN than non-HTN subjects. $F(3, 29) = 4.19$, $P = 0.04$.

Table 3.

Linear Regression Models Relating AL to HTN Status and Inflammatory Markers (TNF α and CRP), Controlling for History of Smoking, BMI, and Plaque Levels.

	Model ($N = 47$)			
	1 ^a	2 ^b	3 ^c	4 ^d
R^2 (adjusted)	0.01 (–0.003)	0.28 (0.22)	0.36 (0.28)	0.43 (0.36)
Hypertension	0.13	0.32*	0.21	0.21
Smoking (history)		0.38**	0.39**	0.27*
BMI		–0.31*	–0.27	–0.42**
Plaque index		0.27*	0.28*	0.18
TNF α (dichotomized) ^e			0.28*	
CRP (ranks) ^e				0.45**

Data are presented as beta weights and are consistent with the notion that the AL-HTN relationship is mediated by TNF α and CRP.

AL, attachment level; BMI, body mass index; CRP, C-reactive protein; HTN, hypertension; TNF α , tumor necrosis factor α .

^aModel 1 shows no association between hypertension and AL.

^bModel 2—which adjusts for history of tobacco smoking, BMI, and plaque index—shows a positive association between hypertension and AL (beta = 0.32, $P < 0.05$) and a notably improved model fit ($R^2 = 0.28$).

^cIn model 3, TNF α (dichotomized data, healthy sites from all subjects) was significantly associated with AL and nullified the significance of the associations between AL with both HTN and BMI.

^dIn model 4, CRP (rank transformed data, healthy sites from all subjects) was significantly associated with AL and nullified the association between AL with HTN.

^eAll subjects, healthy sites only

* $P < 0.05$. ** $P < 0.01$.

The other biomarkers investigated (MMP-8, MCP-1, 8-iso, and IL-1 β) showed no statistically significant associations with HTN; however, their levels were significantly higher in subjects with deep pockets. The association of MMP-8, MCP-

1, 8-iso, and IL-1 β with periodontal disease suggests that they are mainly involved with local gingival microbial infections rather than systemic factors. Previous reports support this view (Kraft-Neumärker et al. 2012; Gupta et al. 2013).

MMP-8 is a collagen-degrading enzyme primarily produced by neutrophils. Inside the cell, it is stored in secondary granules in an inactive form. It is activated in the extracellular environment and plays an important role

in periodontal tissue destruction. It is elevated in deep periodontal pockets and has been reported to vary with clinical measures of gingival inflammation (Teles et al. 2010; Kraft-Neumärker et al. 2012; Pradeep et al. 2013; Sanders et al. 2009).

MCP-1 is a chemokine involved with recruitment of monocytes and T cells to sites of inflammation. Fibroblasts, endothelial cells, and other cells are involved with expressing MCP-1 in inflamed human gingiva. MCP-1 is elevated in the gingival fluid of periodontitis patients, and its levels decrease after periodontal therapy (Gupta et al. 2013; Pradeep et al. 2009). The proportions of MCP-1 in the gingival fluid of hypertensive participants may be related to increased TNF α activity (Bautista et al. 2005).

Isoprostanes are compounds formed from the peroxidation of essential fatty acids. They are potent inflammatory mediators and are used as markers of oxidative stress. Gingival fluid 8-iso levels increase with progression of periodontal disease (Pradeep et al. 2013). IL-1 β is an important inflammatory cytokine produced by activated macrophages and other cells in inflamed gingival tissues. Its levels are elevated in the gingival fluid of periodontitis subjects and are associated with clinical signs of periodontal disease (Figueredo et al. 1999; Sanders et al. 2009).

It is interesting to note that the 6 gingival fluid biomarkers investigated (MCP-1, CRP, TNF α , MMP-8, IL-1 β , and 8-iso) were all detected in clinically healthy gingival sites, suggesting that even in clinically healthy gingiva there is subclinical gingival inflammation. It is noteworthy that in clinically healthy sites, HTN by itself significantly contributed to CRP and TNF α levels. The lack of significant differences in the levels of CRP and TNF α between hypertensive and nonhypertensive subjects with diseased sites suggests that the increased inflammation associated with periodontal disease masks the effect of HTN or a ceiling is reached.

Although our analysis showed that the association between HTN and AL could be partially explained by TNF α and

CRP GCF levels, these findings need to be interpreted with caution. The data are cross sectional and do not support cause-effect interpretations. The sample was limited, and control data examining these relationships in other racial groups were not available. Also, the biomarker data are not normally distributed, and categorical variables were included in the analysis. Nevertheless, the identified relationships are logical. Hypertensive subject have increased levels of CRP and TNF α , even in GCF sampled from healthy gingiva. This may alter the local environment to encourage the multiplication and growth of pathogenic microbial species leading to additional inflammation and increased tissue loss.

In conclusion, individuals with elevated blood pressure show heightened levels of 2 inflammatory mediators in the gingival fluid collected from nondiseased sites, which may exacerbate periodontal tissue damage.

Author Contributions

A. Khocht, contributed to conception, design, data acquisition, analysis, and interpretation, drafted and critically revised the manuscript; T. Rogers, M.N. Janal, contributed to data analysis, critically revised the manuscript; M. Brown, contributed to data acquisition, drafted the manuscript. All authors gave final approval and agree to be accountable for all aspects of the work.

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