IDENTIFICATION OF C-REACTIVE PROTEIN FROM GINGIVAL CREVICULAR FLUID IN SYSTEMIC DISEASE

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ABSTRACT
Periodontitis is associated with elevated C-reactive protein (CRP) in both serum and gingival crevicular fluid (GCF). Although the liver is the primary source of CRP, extra-hepatic production of CRP has been reported. This study aimed to determine whether CRP in GCF is produced locally in the gingivae. **Material and methods** Gingivae and GCF were collected from non-periodontitis and periodontitis sites. Presence of CRP in gingivae was assessed by immunohistochemistry. CRP in GCF was measured using ELISA. Gene expression for CRP in gingivae was determined using real-time polymerase chain reaction. **Results** CRP was found in both the gingivae and GCF. No gingivae had detectable amounts of CRP mRNA. Not all patients with periodontitis had detectable levels of CRP in the GCF. Some non-periodontitis patients had detectable levels of CRP in the GCF. **Conclusions** CRP in the GCF appears to be of systemic origin, and therefore may be indicative of systemic inflammation from either a periodontal infection or inflammatory disease elsewhere. The correlation between levels of CRP in GCF and serum requires validation in future studies.

Key words: *C-reactive protein; inflammation; gingival crevicular fluid*

INTRODUCTION

Periodontal disease has been associated with a number of other inflammatory diseases, including diabetes mellitus, rheumatoid arthritis and cardiovascular disease [1]. The status of many of these systemic inflammatory diseases can be measured using serum markers of inflammation.

Not surprisingly, CRP has been detected in the serum of periodontitis patients, and levels are significantly higher than those of non-periodontitis subjects [2].

CRP has also been detected in the saliva and gingival crevicular fluid (GCF) of periodontitis patients. GCF is a transudate of serum, and as such, contains both serum components and locally produced molecules [3].

As such, the presence of CRP in GCF of periodontitis patients may be due, in part, to the local production of CRP within the periodontal tissues. Local production of CRP would mean that CRP levels detected in the GCF could not be used to make inferences about systemic inflammation [4].

Aim

Therefore, the aim of this study was to determine if CRP detected in the GCF is the result of local production of CRP within the gingival tissues.

MATERIAL AND METHODS

Patients attending the Periodontology Department for periodontal surgery were

invited to participate in the study following written and informed consent. Periodontitis-affected tissue was collected during the periodontal flap surgery carried out for the treatment of persistent periodontal pocketing (PDX5 mm) following initial non- surgical therapy carried out at least 3 months previously. Tissue samples from non-periodontitis sites included gingival hyperplasia without periodontal attachment loss, gingivae resected during crown lengthening surgery or from soft tissue biopsy before tooth extraction. A thorough medical history and smoking history were taken for each patient, as well as questioning whether anti-inflammatory medication, antibiotics or steroids had been taken within the last 6 months. Patients were not excluded on the basis of their medical history or smoking status. Disease classification was in accordance with the American Academy of Periodontology classification (Armitage, 1999) [1], and periodontitis sites exhibited radiographic alveolar bone loss, deepened probing depths and attachment loss to a degree consistent with the rest of the dentition.

Data were entered into a computer database and corrected for implausible data. For the basis of analysis, patients with high blood pressure (including those taking anti-hypertensive medication), cardiovascular disease, high cholesterol, diabetes, arthritis, Crohn's disease or recent antibiotic, anti-inflammatory medication or steroid use were categorized as ''systemically unhealthy'', as these conditions may influence systemic levels of CRP. Patients without these conditions were categorized as "systemically healthy".

RESULTS

A total of 64 samples were taken from 59 individual patients, as detailed in Table 1. Of the 64 samples, 50 were collected from 46 patients with periodontitis, and the remaining 14 were collected from 13 patients who were defined as non-periodontitis, but may have exhibited gingival hyperplasia or gingivitis without periodontal bone loss / attachment loss.

	Total	Periodontitis	Non-periodontitis
Number of samples	64	50	14
Number of subjects	59	46	13
Age (years)*	53.6 ± 15.2	55.5 ± 12.1	46.9 ± 18.5
Female	36/59 = 61%	29/46 = 63%	7/13 = 54%
Male	23/59 = 39%	17/46 = 37%	6/13 = 46%
PD (mm)*	6.4 ± 2.0	7.2 ± 1.3[w]	3.6 ± 1.7
Recession (mm)*	1.3 ± 1.6	1.4 ± 1.5[z]	0.9 ± 2.0
BOP+	45/64 = 70%	41/50 = 82%[§]	4/14 = 29%
GCF volume (mL)*	0.88 ± 0.68	0.98 ± 0.69[w]	0.52 ± 0.55
Current smoker	11/59 = 19%	8/46 = 17%[z]	3/13 = 23%
Systemically healthy	24/59 = 41%	18/46 = 39%[z]	6/13 = 46%
Systemically unhealthy	35/59 = 59%	28/46 = 61%	7/13 = 54%
Cardiovascular disease	3	1	2
Diabetes	9	8	1
Arthritis	8	7	1
Crohn's disease	1	0	1
Antihypertensive medication	16	13	3
Cholesterol lowering medication	12	10	2
Anti-inflammatory medication (including aspirin)	17	14	3
Antibiotics	2	1	1
Samples with CRP detectable in GCF	26/64 = 41%	19/50 = 38%[§]	7/14 = 50%

Table 1. Subject, sample demographics and clinical parameters in the study population

The pocket depth for the periodontitis sample sites ranged (SD ± 1.3 mm). This was significantly deeper than that measured for the non- periodontitis samples with a mean of 3.6 (SD ± 1.7 mm) (Mann–Whitney test, po0.0001). Similarly, the percentage of sites that bled upon probing (measured following GCF collection) was significantly higher for the periodontitis sites, compared with the non-periodontitis sites (82.0% versus 28.6%, Fisher's exact test, p 5 0.0003). The mean GCF volume for the periodontitis samples was 0.98 (SD ± 0.69) mL, which was also significantly different from the non-periodontitis samples at a mean of 0.52 (SD ± 0.55) mL (Mann– Whitney test, p 5 0.0159).

Among the periodontitis samples, 39.1% were from systemically healthy patients without any known medical conditions or taking any medications, while among the non-periodontitis samples, 46.2% were systemically healthy, and this difference was not significantly different (Fisher's exact test). The two most common conditions experienced among this sample population were currently taking anti-hypertensive medication or anti-inflammatory medication (including aspirin).

Detection of CRP in GCF samples

The subject and sample demographics and clinical parameters for samples with detectable CRP in GCF, in comparison with the total study population are described in Table 2.

	Total	Periodontitis	Non-periodontitis
Number of samples	26/64= 41%	19/50 = 38%*	7/14 = 50%
BOP+	16/45 = 36%^{w}	14/41 = 34%*	2/4 =50%
BOP -	10/19= 53%	5/9 =56%	5/10 =50%
GCF volume (µL)	0.79 ± 0.68	0.90 ±0.70*	0.51 ± 0.56
Systemically healthy	10/24= 42%^{z}	6/18 =33%*	4/6 =67%
Systemically unhealthy	16/35= 46%	13/28 =46%	3/7 =43%
Current smoker	4/11 = 36%§	2/8 =25%	2/3 =67%
Former or never smoker	22/48= 46%	17/38 =45%	5/10 =50%

Table 2. Proportion of samples with detectable C-reactive protein in GCF compared with total sample population

Approximately, 41% of the 64 GCF samples tested by ELISA had detectable CRP. Numerically, a higher percentage of non-periodontitis GCF samples had detectable CRP compared with the periodontitis GCF samples, but this difference was not statistically significant (50% versus 38%, Fisher's exact test). Bleeding on probing (BOP) did not appear to have a positive effect on CRP detection in the GCF with only 35.6% of BOP positive sites subsequently found to be positive for CRP, compared with 52.6% of BOP negative sites, which was not a significant difference (Fisher's exact test). In addition, there were no significant differences in the proportions of BOP positive sites with detectable CRP in the GCF between the periodontitis compared with the non-periodontitis groups (34.1% versus 50.0%, Fisher's exact test). Also, there was no significant difference in the volume of GCF for those with detectable CRP compared with the total study population (Mann – Whitney test, data not shown). Systemic health status also did not appear to have a significant influence on CRP detection in the GCF with similar proportions of detection in both systemically healthy and unhealthy groups. Overall, 10 of the 24 (41.6%) systemically healthy patients and 16

of the 35 (45.7%) patients who were not systemically healthy had detectable CRP in GCF, and this was not statistically significant (Fisher's exact test).

The 10 systemically healthy patients included two cur- rent smokers and two of the systemically unhealthy patients were also current smokers, so that overall four of the 11 (36%) current smokers had detectable CRP in the GCF. Only three of the patients with detectable CRP in the GCF were former smokers, and 19 were never smokers. Owing to the small number of current smokers in the study, statistical significance of smoking on CRP detection was not determined in the current study. However, the proportion of current smokers with detectable CRP was not significantly different from the proportion of former or never smokers with detectable CRP (36.3% versus 45.8%, Fisher's exact test).

Table 3 describes the subject and sample demographics and clinical parameters for only the samples with detect- able CRP in GCF. Of the 26 samples with CRP detectable in the GCF, 73.1% were periodontitis samples, in keeping with the higher proportion of periodontitis samples in the total sample collection.

	Total	Periodontitis	Non-periodontitis
Number of subjects	26	19	7
Number of samples	26	19/26 = 73%	7/26= 27%
BOP+	16/26 = 62%	14/19 = 74%*	2/7 =29%
GCF volume (μL)[w]	0.79 ± 0.68	0.90 ± 0.70*	0.51± 0.56
Systemically healthy	10/26 = 38%	6/19 = 32%*	4/7 =57%
CRP amount (pg)[w]	0.12 ± 0.18	0.13 ± 0.21*	0.08± 0.09

Table 3. Subject and sample demographics and clinical parameters in the samples with detectable C-reactive protein in GCF

Seventy-four percent of the CRP positive periodontitis GCF samples and 28.6% of the non-periodontitis GCF samples were taken from sites that subsequently bleeding upon probing, this difference approached statistical significance at p 5 0.0687 (Fisher's exact test), and overall 61.5% of the sites with detectable CRP subsequently bleeding upon probing. These figures reflect the proportions of BOP sites in the periodontitis and non-periodontitis samples seen in the total study population. There were no significant differences in the volume of GCF between periodontitis and non- periodontitis samples (Mann–Whitney test).

DISCUSSIONS

In the current study, as in previous studies, CRP has been detected in the GCF and periodontal tissues of both non-periodontitis and periodontitis sites and subjects.

However, the origin of this protein in GCF has not been investigated previously [5].

Based on the absence of CRP mRNA in the periodontal tissues, it can be deduced that the origin of CRP in GCF is not from the local periodontal tissues. Further support for the absence of local production of CRP is that within the periodontal tissues the distribution of CRP was diffuse throughout the connective tissue and was not cell-associated.

Therefore, the presence of CRP in the GCF and periodontal tissues appears to be of systemic origin. The main source of CRP is acknowledged to be the liver, and although several other tissues have recently been shown to produce; these are regarded as having minimal contribution to serum levels of CRP.

Systemic CRP detected in GCF and periodontal tissue may be a result of systemic inflammation resulting from disease

elsewhere in the body as well as systemic inflammation induced by periodontitis [6].

Indeed, in this study the majority of patients with detectable CRP had either periodontitis or systemic inflammatory disease, and it is very possible that the remaining patients had an undiagnosed systemic inflammatory disease or a recent infection, which may explain the detection of CRP, given that it is not of local origin. In the current study, serum levels of CRP were not analysed [7].

While the collection of serum samples would have enabled a correlation between GCF and serum levels of CRP, the aim of this study was primarily to determine if CRP was locally produced in the periodontal tissues and not to correlate CRP in the GCF with CRP in the serum. Collecting serum samples was not necessary to determine if CRP was produced locally, and GCF samples were primarily included to assess whether CRP was present or absent in the periodontal tissues. As GCF is a transudate of serum, and our results establish that CRP is not produced locally in the periodontal tissues, CRP in the GCF must be derived from serum CRP, and may be indicative of systemic inflammation [8, 9]. However, further studies are needed to correlate levels of CRP in the GCF with that of serum before GCF could be considered to be suitable as a source for the non-invasive assessment of the degree of systemic inflammation in both periodontitis and non-periodontitis patients.

CONCLUSIONS

In conclusion, the findings of this study indicate that CRP detected in the GCF and periodontal tissue is not of local origin. This implies that elevated serum CRP in periodontitis patients is not due to local production but could be indicative of systemic inflammation, either as a result of periodontal infection or systemic disease. As GCF is a serum transudate, we propose that it may be considered as a substitute source with which to assess systemic inflammation as measured by CRP.

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