Correlation between crevicular C-reactive protein level with its serum level in chronic periodontitis patients

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ABSTRACT

Background: The main purpose of this study is to find if there is any correlation between the level of C-reactive protein (CRP) in gingival crevicular fluid with its serum level in chronic periodontitis patients and to explore the differences between them according to the probing depth.

Materials and methods: Forty seven male subjects enrolled in this study. Thirty males with chronic periodontitis considered as study group whom further subdivided according to probing depth into subgroup 1 with pocket depth ≤6mm, subgroup 2 with pocket depth >6mm. The other 17 subjects considered as controls. For all subjects, clinical examination where done for periodontal parameters plaque index (PLI), gingival index (GI), bleeding on probing (BOP), probing pocket depth (PPD) and clinical attachment level (CAL). The gingival crevicular fluid (GCF) were collected using filter paper size 30 from gingival sulcus of the controls and from (138) pocket site (75 sites > 6mm. and 63 ≤ 6mm.). The weight of the GCF was measured by reading the difference in the weight of filter paper before and after absorption of GCF. Crevicular level of CRP was measured calorimetrically. The serum level CRP was measured using latex test.

Results: Highly significant difference in the weight of GCF, crevicular and serum level of CRP between chronic periodontitis and control groups. Subgroup 2 got higher scores of weight of GCF and positive record of crevicular and serum CRP compared with subgroup 1 with a non-significant difference. A highly significant difference in the number of sites with positive crevicular and serum CRP compared to the negative number between chronic periodontitis and control groups also between subgroup1 and subgroup 2.

Weight of GCF gets a negative significant correlation with GI at control group and subgroup2. Serum level of CRP exhibits a negative significant correlation with PLI for chronic periodontitis and control group and positive significant correlation for GI at subgroup 1. The crevicular CRP get significant negative correlation with GI of subgroup 1.

Conclusions: Crevicular fluid is very good marker for the degree of inflammation of the periodontal pocket. The crevicular level of CRP may be considered as a good tool for estimating the systemic effect and predictor for the effect of periodontitis on the general health and the correlation of crevicular with serum CRP aid to high light this effect.

Keywords: Crevicular C reactive protein, chronic periodontitis, gingival fluid. (J Bagh Coll Dentistry 2014; 26(3):84-88).

الخلاصة

الهدف الرئيسي من هذه الدراسة هو معرفة تركيز البروتين الارتكاسي في السائل اللثوي ومقارنته مع تركيزه في مصل دم مرضى النسغاغ المزمن بالمقارنة مع المجموعة الضابطة ايضا تحاول الدراسة ايجاد علاقات بين تلك التراكيز ومؤشرات ماحول الاسنان السريرية في مجموعة الدراسة والمجموعة الضابطة. توصلت الدراسة الى انه هناك فرق واضح في التراكيز بين كلا المجموعتين وكذلك فان مستوى البروتين الارتكاسي يختلف باختلاف شدة الاصابة بمرض النساغ المزمن وخلصت الدراسة الى ان مرض النساغ المزمن مع مؤشراته المناعية قد يعكس صورة تاثير هذا المرض على الصحة العامة

INTRODUCTION

Periodontitis is a group of inflammatory diseases of teeth supporting tissues. The spreading of the inflammatory process from the gingival deep into periodontal tissues may lead to the destruction of the periodontal ligament and considerable bone loss in the alveolar process. A large part of therapy for periodontitis is eliminating inflammation. This is not only to preserve periodontal tissues but also to eliminate an oral source of inflammation contributing to over all systemic health [1].

Recent efforts have focus on periodontitis as a potential trigger for systemic inflammation [2]. Serum C - Reactive proteins (SCRP) level is often elevated in subjects with periodontitis compared to non – periodontitis subject [3]. This acute phase protein is produced in the liver in response to inflammatory or infections stimuli and act as inflammatory markers C-reactive protein induces monocytes and or macro phages to produce tissue factors which stimulate the coagulation pathway and increases blood coagulability. CRP also stimulates the complement cascade, further exacerbating inflammation [1,4]. CRP level is positively influenced by conventional periodontal treatment and may significantly improve the systemic condition [5].

Gingival crevicular fluid (GCF) is an exudates of varying composition seeps into gingival crevice, or periodontal pockets around teeth, it is a complex mixture of serum, inflammatory cells, connective tissue, epithelium, and microbial flora inhabiting the gingival margin or the sulcus or pocket. These substances possess a great potential for serving as indicators of periodontal disease and healing after therapy [6].

The present study aimed to detect the weight of GCF and assess the level of CRP in GCF and serum and compare between them in chronic periodontitis patients compared to controls. Also aimed to determine if the crevicular CRP is the result of its local production within the periodontal pocket by comparing its level with different pocket depths and compared to its serum

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level and finally to correlate the immunological parameters (weight of GCF, crevicular and serum CRP) with clinical periodontal parameters PLI, GI, BOP, PPD, CAL for all studied groups and subgroups.

MATERIALS AND METHODS

Forty seven male subjects attending the Department of Periodontics at College of Dentistry/ University of Baghdad were invited to participate in the study following written and informed consent. The subjects were divided to study and control groups.

The study group composed of 30 subjects with an age ranged between 35-50 years with chronic periodontitis (CPG) according to the international classification system for periodontal disease [7]. The total numbers of the examined sites equal to 138 pocket sites. The study group further subdivided according to their pocket depth into 2 subgroups. Subgroup 1; included 16 males with 63 sites of PPD ≤ 6 mm., subgroup 2; included 14 males with 75 sites of PPD > 6mm.

The control group (CG) consisted of 17 subjects with clinically healthy periodontium with an age ranged between 35-45 years. All subjects were non-smoker, and systemically healthy according to their medical history. All didn't receive any periodontal treatment or take local or systemic antibiotics or anti-inflammatory medications within the previous three months.

Periodontal assessments include the following indices:-

1. Plaque index system PLI according to Silness and Loe 1964 [8]
2. Gingival index GI by Loe 1967 [9]
3. Bleeding on probing BOP expressed as percentage of site with BOP [10] which are either positive (+ve) or negative (-ve).
4. Probing pocket depth PPD measured from the gingival margin to the most apical penetration of periodontal probe [7]. The mean PPD of each patient of the study group determine his location in either subgroup 1 or 2
5. Clinical attachment level (CAL): Measured from the C.E.J. to the deepest point of inserted probe tip.

All measurements were done for all the present teeth except 3rd molar [7] by Williams' periodontal probe

Collection of crevicular fluid

The collection of GCF was done on a day other than the day of clinical examination. After a thorough supra-gingival scaling, the teeth were carefully dried before the collection of the fluid using pre-weighted filter papers size 30 which were gently inserted into the selected pocket for the study group and to the depth of gingival sulcus in the control group. The paper left for 30 second [11], and re-weighted using a chemical balance after their removal.

Each paper placed in tube containing 0.3 ml. normal saline then transferred and stored at -20º C. At the day of analysis the sample were centrifuged at 10.000 rpm /20min. The supernatant was used for assessment of CRP calorimetrically. If the concentration of crevicular CRP (C.CRP) ≥ 6Pg/ml. this is considered as a + ve finding.

Serum collection

Identification of the concentration of serum CRP (S.CRP) was done using latex test.

2 ml. venous blood was withdrawn from each subject .The blood then centrifuged at 3000 r.p.m. for 5min. The supernatant was harvested for further analysis by agglutination test for detection of serum CRP. If the agglutination took place within the first seconds, the positive reading was considered strong + ve. The normal concentration of serum CRP ranged between 2-12 mg/l.

Statistical analysis

The mean values and standard deviations of the measured parameters were obtained and groups' differences were assessed using t-test. The correlation between the crevicular and serum level of CRP with clinical parameters was examined by Pearson's correlation analysis. Chi square test was used to assess the differences in the numbers of the +ve records with the –ve records. The probability values greater than 0.05 were considered non-significant

RESULTS

A total of 138 periodontal pocket sites were included in this study from chronic periodontitis group. Pocket depth ≤ 6mm were equal to 63 sites while pockets greater than 6 mm were 75 pockets. Table 1 represented the descriptive data of the CPG, CG and subgroup 1&2 for PLI, GI, PPD, CAL and BOP.

Table 2 represented the descriptive statistics of the immunological parameters including weight of GCF, crevicular and serum mean number of positive CRP for CPG, CG and for both subgroups. It appears that the weight of GCF increase in CP group (0.6±0.166) over the CG (0.14±0.05).

Also the mean number of positive results/subject of S. CRP is higher in CPG (0.72±0.45) than CG (0.17±0.39). The C. CRP

equals to (0.55±0.5) among CP group while no one in the CG revealed a positive crevicular CRP.

A highly significant difference in the level of S. CRP, C. CRP and weight of GCF between CPG and CG was presented in table 3.

When comparing the difference in the weight of GCF and the positive results of crevicular and serum CRP between the two subgroups, it appears that all the immunological parameters carry highest scores within subgroup 2 compared to those in subgroup1 with a non-significant difference. A significant difference appear in the weight of GCF between subgroup 1&2 (Table 2).

Table 1: Descriptive statistics of clinical parameters for all studied groups (mean± SD)

Parameter	CPG	CG	Subgroup 1	Subgroup 2
PLI	1.272±0.422	0.411±0.126	1.3±0.408	1.23±0.45
GI	1.179±0.225	0.38±0.09	1.18±206	1.169±0.256
PPD	5.241±1.09		4.37±0.5	6.307±0.480
CAL	6.03±1.592		5.68±1.77	6.46±1.26
BOP	0.552±0.506	0	0.5±0.516	0.615±0.506

Table 2: The descriptive statistics of the immunological parameters for the studied groups (mean ±SD)

Parameter	CPG	CG	Subgroup 1	Subgroup 2
Weight/GCF	0.6±0.16	0.14±0.5	0.58±0.17	0.62±0.15
C.CRP	0.55±0.5	0	0.44±0.51	0.69±0.48
S.CRP	0.72±0.45	0.17±0.39	0.68±0.47	0.76±0.43

Table 3: The significant differences in the mean number of positive results of CRP/subjects and weight of GCF (inter groups and subgroups comparison)

Immunological parameters	CPG/CG	Subgroup 1/subgroup2
Weight GCF	t-test = 2.09	t-test = 2.05
	P value = 0.001 HS	P value = 0.507 NS
C.CRP	t-test = 2.01	t-test = 2.06
	P value = 0.000 HS	P value = 0.18 NS
S.CRP	t-test = 2.02	t-test = 2.05
	P value = 0.0001 HS	P value = 0.63 NS

Correlation of clinical with the immunological results among all studied groups

Correlation of the clinical parameters in all studied groups and subgroups with the immunological findings are shown in table 4.

For CPG, the PLI and GI showed negative correlation with the crevicular and serum levels of CRP while they showed a positive correlation with the weight of GCF. BOP showed a non-significant direct correlation with the crevicular and serum CRP levels and the weight of GCF.

A non-significant negative correlation of the CAL appears with weight of GCF and the serum CRP and non significant positive correlation with crevicular CRP. While PPD correlated non-significantly with all immunological parameters within CPG in positive direction.

For the subgroup I, PLI showed a non-significant positive correlation with the weight of GCF and S.CRP, and a significant negative correlation with crevicular CRP.

GI presented a significant positive correlation with crevicular CRP and significant negative with serum CRP, and it correlated non-significantly with the weight of CGF. BOP correlated positively with weight of GCF, serum and crevicular CRP. CAL correlated non-significantly with all immunological parameters. The probing depth got a non-significant positive correlation with weight of GCF, C.CRP and S.CRP.

For subgroup 2, PLI and GI showed a non-significant correlation with the crevicular and serum CRP. PLI correlated significantly with the weight of GCF.

BOP showed positive non-significant correlation with weight of GCF and C. CRP while it correlated non-significantly with S.CRP.

The CAL showed non-significant negative correlation with the weight of GCF but the correlation was non-significant positive with the crevicular and serum CRP. The probing pocket depth has a weak non-significant positive correlation with the level of CRP in crevicular

fluid and serum while the correlation was negative with the weight of GCF

For CG, the weight of GCF correlated non-significantly with PLI and significantly with GI. S.CRP got a significant negative correlation with PLI and non-significant positive with GI.

The correlations between the immunological parameters within the two subgroups are shown in table 5. The correlation between crevicular CRP and serum CRP level within subgroup 1 and 2 appears to be weak non-significant positive correlation. On the other hand, the weight of GCF correlated non-significantly with crevicular CRP in both subgroups.

Table 6 represented the significance difference in the numbers of the positive and negative scores of CRP for subgroup 1 and 2 using Chi square test. The data showed that there is a non-significant difference between the number of subjects who develop a positive finding of either crevicular or serum CRP compared to those with negative findings.

Table 4: Pearson correlation between the clinical and immunological parameters for all studied groups

	Weight GCF				S. CRP				C. CRP			
	CP	CG	Subg 1	Subg 2	CP	CG	Subg 1	Subg 2	CP	CG	Subg 1	Subg 2
	r/sig.				r/sig.				r/sig.			
PLI	0.62 S	0.2 NS	0.65 NS	0.64 NS	-0.04 S	-0.04 S	0.14 NS	-0.25 NS	-0.23 NS		-0.22 S	-0.23 NS
GI	0.22 NS	-0.007 S	0.50 NS	-0.02 S	-0.16 S	0.19 NS	0.03 S	-0.37 NS	-012 S		-0.01 S	-0.22 NS
BOP	0.08 NS		0.11 NS	0.016 NS	0.06 NS		0.13 NS	-0.06 NS	0.60 NS		0.63 NS	0.50 NS
CAL	-0.20 NS		-0.21 NS	-0.34 NS	-0.13 NS		-0.44 NS	0.36 NS	0.15 NS		-0.06 NS	0.40 NS
PPD	0.06 NS		0.16 NS	-0.54 NS	0.21 NS		0.24 NS	0.37 NS	0.40 NS		0.36 NS	0.04 NS

Table 5: Correlation of immunological parameters with each other

Parameters	Subgroup1(r/sig.)	Subgroup 2(r/sig.)
Weight/C.CRP	0.09/NS	-0.34/NS
S.CRP/C.CRP	0.32/NS	0.43/NS

Table 6: Comparison of +ve/-ve numbers for the subgroup1 and 2

Parameters	Subgroup 1 N=16		Subgroup 2 N=14		X^2	df	Sig.
	+/ %	-/ %	+/%	-/%			
C. CRP	7/43.0	9/64	9/64.2	5/35.7	1.88	1	0.17 (NS)
S. CRP	11/68.7	5/31.2	10/71.4	4/28.5	0.4	1	0.62 (NS)

DISCUSSION

The crevicular fluid provides a non-invasive access method to the periodontium. The marker in GCF is considered a good method in the determination of a person's risk for periodontal disease [12].

The increase in the weight of GCF in the present study among the CP patients compared to controls also represented in other studies who studied the volume of GCF which found to be associated with diseases state compare to healthy [13].

On the other hands its clearly obvious that GCF increase in the severity of inflammation in term of pocket depth and this in agreement with some studies [13,14].

C-reactive protein is a trace protein in the circulation of healthy subject with a median concentration of 1mg/l. This concentration can be increased 100 fold or more in response to injury, infection or inflammation [15,16].

In patients with periodontitis, the periodontal pathogens presence in periodontal pocket/ sulcus can elicit some source of bacterial products that could lead to activation of liver through systemic antibody response and so increase in CRP production, that's why an increase in S.CRP was seen in CP patients compared to controls and the level of S.CRP increased with the increase in the pocket depth compared to controls [17].

A small elevation in CRP elicited by periodontal disease might be considered potential

risk factor for systemic disease as cardiac disease. The elevated level of CRP in GCF of CP patients compared to control who registered no CRP in their GCF corresponded with findings of Macovei et al. [18].

As no one of the subjects in control group registered a positive reading of C.CRP, this means that the production of CRP in GCF is in response to local inflammation within the periodontium in addition to the systemic effect. On the other hand, the positive reading of S.CRP with C.CRP within CP group reflects the effect of undiagnosed systemic condition that could aid in elevation level of CRP in GCF i.e. effect of periodontitis to elicit systemic inflammation [18,19].

The difference in the correlation results of clinical periodontal parameters with the immunological parameters may be related to methodology and number of examined subjects in this study. Also the non-significant correlation of crevicular level of CRP with its serum level in accordance to probing depth gives an idea about the presence of such correlation which needs further analysis using larger sample size and more sensitive method of analysis.

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