Determination of Interleukin-1 (IL-1) and Interleukin-6(IL6) in Gingival Crevicular Fluid in Patients with Chronic Periodontitis

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Abstract:
Background*: Gingival crevicular fluid (GCF) is an exudate that can be collected from the sulcus or periodontal pocket. It contains a variety of substances including immunoglobulin, microorganisms, toxins , cells, and lysosomal enzymes and markers Analysis of GCF is a non-invasive method to study the host response of the periodontium and inflamed tissues. It has been consider as a promising medium for an early indicator for early detection of inflammatory cytokines that play a major role in destruction of periodontal tissue.*

Subjects and methods: *In the present study (52) males patients were enrolled with an age ranging from (30-55) years. The sample were divided into two main groups (26) healthy control and (26) patients with chronic periodontitis (CP). All were from attendants to department of Periodontics ,School of Dentistry, University of Sulaimani .All subjects were in good general health and had not received previous periodontal therapy or taken antibiotics ,or anti-inflammatory drugs in the three months before the study . Clinical Periodontal Parameters include Plaque index(PLI) ,Gingival Index(GI) , bleeding on Probing(BOP), probing Pocket depth(PPD) and clinical attachment level (CAL). The gingival crevicular fluid was collected from each subject by using paper point (size30)which was inserted into the gingival crevice and kept in place for30seconds.The fluid volume was determined by using Periotron (Harco6000,USA).The concentration of interleukin-1 and the Interleukin 6 (IL-6) in gingival crevicular fluid was quantified by - sensitivity enzyme linked immunosorbent assay(ELISA) .The concentrations of interleukin-1 and the Interleukin6 (IL-6) in gingival crevicular fluid was measured in(pg/µl).*

Results*: There were high significant difference between chronic periodontitis and control group in clinical parameters [Plaque index (PLI) ,Gingival Index(GI) , Bleeding on Probing(BOP%]), Pocket depth(PPD)] p-value (0.000). The concentration of interleukin- IL -1ß in GCF was higher in chronic periodontitis group (208.72±52.25) than control group (49.04±16.73). In addition, the concentration of interleukin-6 (IL-6) in GCF was higher in chronic periodontitis group ((9.76±2.98 pg/µl) than control group(3.13 ±1.71).Moreover, in chronic periodontitis group the mean of probing pocket depth group (5.74±1.47) and the mean of clinical attachment loss (3.46±1.51).*

Conclusion*: In GCF the concentration of interleukin -1 (IL-1)and interleukin-6 (IL-6) (pg/µl) were higher in chronic periodontitis group than in control group. They can be regard as diagnostic marker which give information about progression of periodontal disease.*

keywords: *cytokine , of interleukin- IL-1 (IL-1ß) ,Interleukin-6 (IL6) , Gingival crevicular fluid ,Chronic Periodontitis*

I. Introduction

Periodontal disease (PD) is the Second most common oral disease in the human being .Its' prevalence in adults differs from 10% to 60% depending on the criteria of diagnosis **(1)**. Chronic multifactorial polymicrobial infection which is characterized by that result in destruction o f periodontium(**2**).Environmental, genetic factors and immune system take part in process of inflammation **(3)**. Periodontitis results in local increases in levels of pro-inflammatory cytokines which are considered to play an essential role in chronic periodontitis inflammatory process**(2)**.

Cytokines are small polypeptides with a wide range of inflammatory, metabolic and immunomodulatory properties **(4)**.They are manufactured by macrophage, lymphocytes, monocyte, dendritic cells, lymphocytes, neutrophils, endothelial cells and fibroblasts **(4;5)**.Cytokines are the mean of communication between immune and non-immune cells **(6)**. Inflammatory mediators are important to the pathogenesis of periodontal diseases and may be used as diagnostic markers **(7)**. Interleukin (IL)-1 is present in two active forms,IL-1 andIL-1 .Both are potent Proinflammatory molecules and are the main components of osteoclast-activating factor **(8)**.Interleukin (IL)-1 is produced by Macrophages and marrow stromal cells .It stimulates bone resorption and participate in pathological conditions with bone loss **(5)**.Interleukin-1 (IL-1) which is a vital Proinflammatory cytokine play a major role in inflammation and bone resorption, therefore it becomes an

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important parameter in periodontal research. Analysis of IL-1 levels in GCF is one manner used to estimate its local IL-1 concentrations **(9)**.The increased concentrations of IL-1 in GCF which was collected from sites with periodontal disease, compared to its concentrations in healthy periodontium may manifested the close relationship between IL-1 concentration and progression of periodontal disease **(10;11;12)**.Therefore , measurement of IL-1 , , in gingival crevicular fluid (GCF) or tissues adjacent to periodontitis-affected sites in patients, has been suggested as a sensitive and remarkable method in monitoring severity of periodontal disease **(11)**. Interleukin-6(IL-6)is pivotal cytokine involved in the regulation of host response to infection and tissue injury **(13)**.It is synthesized b y different cells, for instance ,monocytes ,fibroblasts**(14)** osteoblasts**(15)**and vascular endothelial cells **(16)**in response to inflammatory challenges**(17)** It plays a fundamental role in differentiation of B-cell(18) and in T-cell proliferation(19).

Synergism between IL-6 and interleukin-1 (IL-1), induces bone resorption **(15)**. Interleukin 6 (IL-6) is critical parameter in periodontal research because of its' effect in inflammation and bone resorption by stimulation activity of the osteoclasts **(20,21)**.The expression level of Interleukin-6(IL-6)has been related to the severity of periodontal disease**(22)** and age **(23)**.

Gingival crevicular fluid (GCF) is an inflammatory exudate that infiltrate into gingival sulcus or periodontal pockets (24).It can be collected from healthy gingival sulcus , although only in small amount. GCF represent the transudate of gingival tissue interstitial l fluid produced by an osmotic gradient In the healthy periodontium, (25). It contains different substances including: immunoglobulin, microorganisms, toxins, cells, and lysosomal enzymes and markers (26).

Cytokines, have been detected in gingival crevicular fluid (GCF) (27) and presence of such constituents can be of effective value in evaluating periodontal disease condition or outcomes of periodontal treatment (28;29).The collection of GCF is non-invasive process and the analysis of specific components in the GCF supplies a quantitative biochemical marker for the estimation of the local cellular metabolism that exhibits periodontal health condition (30). Participation of GCF fluid-derived biomarkers combined with periodontal pathogens and clinical measurement provides a remarkable method for differentiation of (PD) periodontal disease progression (31).

The present study was conducted to determine the levels of Interleukin 1 ß (IL -1ß) and Interleukin-6 (IL6) in gingival crevicular fluid and correlate their levels with clinical parameters in patients with chronic periodontitis when compared to subjects with healthy periodontium.

II. Aims of the study

To determine the levels of Interleukin Interleukin-**1** (IL-1) and Interleukin-6 (IL6) in patients with chronic periodontitis when compared to subject with normal periodontium.

To correlate the levels of Interleukin -1 ß (IL -1ß) ,and Interleukin-6 (IL6) with the clinical parameters [plaque index(PLI),gingival index(GI), probing pocket depth(PPD) and clinical attachment loss (CAL) .

III. Materials and Methods

III. 1 Subjects

Fifty two (52) males patients , (26) healthy control patient and (26) patients with chronic periodontitis(CP) with an age ranging from(35-55)years were recruited at department of periodontics, School of Dentistry, University Sulaimani .All individuals were in healthy systemic condition and had not received any p a s t periodontal treatment or taken antibiotics, immunomodulatory or anti-inflammatory medicine in the three months before the study. Official ethics review committee approval for the research was gained. Each patient was supplied with a written explanation of the purpose of the study and signed consent.

III. 2 Exclusion Criteria

Patient taken systemic antibiotic therapy or anti-inflammatory treatment remedy with in the last three months subjects with any systemic disease including diabetes, bacterial, viral and fungal infections, Smoking, alcohol drinking, and individual with less than twenty teeth were excluded from this research.

III. 3 Design of the Study

All the individuals were informed about the aim of the study and they were free to agree or refuse to be take part in the research and they should signed consent .They were subjected to a questionnaire including: name ,age, full medical, dental and drug history, and if they smoked or drank alcohol. Following this full examinations, GCF collection and registration of clinical periodontal parameters (PLI, GI,BOP, PPD and CAL) were performed.

III . 4 Clinical Periodontal Parameters

All teeth except third molars were assessed for periodontal clinical measures.

III . 4 .1 Plaque index (PLI)

The methods of assessment of bacterial dental plaque , according to the plaque index (32)by using a straight sharp explorer and record the amount of plaque on of each tooth for four surfaces , buccal (labial) lingual (palatal) , mesial and distal surfaces .

III. 4 .2 Gingival Index (GI)

The gingival inflammation at the four surfaces(buccal (labial) lingual (palatal) , mesial and distal)of each tooth was assessed using the criteria of gingival index (GI)(33).

III. 4 .3 Bleeding on Probing (BOP)

William periodontal probe markings at (1,2, 3,5,7,8,9 and10mm) was inserted to the bottom of the periodontal pocket or gingival sulcus for four surfaces (labial/ buccal ,lingual/palatal, mesial and distal)of each tooth and it was moved gently along the tooth(root) surface .In case of bleeding emerges within 30 second after probing the site was given as positive(+) score(1) and a negative (-) score (0) for" 0" for site of absence of bleeding according to(Gingival Sulcus Bleeding Index) (34).

III . 4 .4.Probing Pocket Depth (PPD)

By using William periodontal probe the distance in millimeters from the gingival margin to the base of gingival sulcus or p e r i o d o n t a l pocket was recorded .It was inserted into gingival sulcus or pocket as close as possible to the long axis of the tooth at four surfaces of each tooth (labial/ buccal ,lingual /palatal, mesial and distal) surfaces ,no pressure was used to insert periodontal probe (35).

III . 4.5 Clinical attachment level (CAL)

The distance between the cemento-enamel junction[CEJ] in an apical direction and the base of gingival sulcus or pocket will measure to the nearest millimeter by using William s graduated periodontal probe which inserted into the buccal (labial),lingual (palatal) ,mesial and distal surfaces for each tooth (36) .

III . 5 Detection of interleukin- 1 (IL-1)) and Interleukin-6 (IL6) in the GCF

The levels of interleukin- 1 (IL-1)) and Interleukin-6 (IL6) in the GC F were determined by using enzyme-linked immunosorbent assay (ELISA)(Micro plate ELISA reader device (Human, Germany).

III . 6 Collection of Gingival Crevicular Fluid.

Test sites were chosen from maxillary teeth to avoid contamination with saliva .GCF samples were obtained only from the buccal sites to ensure accessibility and isolation. All participants were instructed not to eat anything or brush their teeth for 1 h before GCF collection to avoid interference with the GCF volume. Prior to the GCF collection, immediately following isolation of tooth with cotton rolls, , supragingival plaque was removed using curette without touching the marginal gingiva. Sites were gently dried with a short blast of air directly through the contact (not into the sulcus/pocket) (1)and paper point size(30)for each examined site was inserted into gingival sulcus ,until mild resistance was felt and waskepttherefor30s.(37;38;39) as shown in (fig.1) .paper point contaminated by bleeding were discarded. The amount of GCF collected was quantitated by using Periotron 6000(Harco6000), USA) as shown in (Fig.2), the paper point placed following collection of GCF placed in eppendroffs tubes contain (300 microliter) phosphate buffer saline. Elution of GCF from paper point by centrifugation at 3000 rpm Centrifuge by using (Heraeus Labofuge 200, U.S.A) for15 minutes and then the paper point was removed. The GCF sample kept at (-40 ˚C) till analysis by Enzyme linked immunosorbent assay(ELISA).

Statistical analysis

Data were analyzed using SPSS (statistical package of social science) software version 19. In this study the following statistics were used:

1. Descriptive statistics: including means, standard deviations, standard errors and statistical tables.

2. Inferential statistics: including:

a) Independent sample t-test: to compare the measured variables between control and study groups.

b) Pearson's correlation coefficient test (r): to test the relation between the measured variables in each group.

In the statistical evaluation, the following levels of significance are used:

Non-significant NS P >	0.05
Significant S 0.05	P > 0.01
Highly significant HS P	0.01

IV. Result

IV.1Descriptive statistical results

The descriptive statistical results of clinical parameters were found that the mean of PLI was higher in CP group (0.93±0.39) than control group(0.27±0.09). Also, the mean values of GI was higher in CP group(1.15±0.47) group) than control group (0.28±0.10).In addition, the mean percentages of bleeding on probing sites in CP group(42.09±28.30)were higher than in control group (1.44± 1.91). Moreover ,the volume of GCF was higher in CP (119.62±49.94) than in control group(33.58±10.66).Furthermore, there were high significant difference p-value(0.000) in the mean of concentration of IL-1B (pg/µl) in GCF between CP (208.72±52.25pg/µl)) and control group (49.04±16.73)pg/µl). There were high significant difference in concentration of interleukin 6 (IL-6)(pg./µl) in GCF p-value(0.000) between CP(9.76± 2.98) and control group (3.13±1.71) as shown in table (1).The mean of probing pocket depth (5.74 ±1.47) in CP group and the mean of clinical attachment loss (3.46±1.51) as shown in table (2).

IV.2 Correlation among variables in control group
Table (3) clarifies the correlation among variables in control group.
IV.2 .1Correlation between PLI and other variables
There was weak positive none significant correlation between PLI and the level of IL6 in GCF r
(0.014) , p-value (0.947) . Also , there was weak positive none significant correlation between PLI and the level of interleukin -1 (IL-1) in GCF r (0.253),p-value (0.212). In addition, there was weak positive non- significant correlation between PLI and volume of GCF r (0.290) , p-value (0.151) .Moreover, there was positive weak none significant correlation between PLI and BOP% r (0.253) ,p-value (0.213). While There was positive moderate high significant correlation between PLI and GI r (0.487) ,p-value (0.012).
IV.2 .2Correlation between GI and other variables
There was weak positive none significant correlation between GI and BOP% is r (0.327) , p-value (0.103). Also, There was weak positive none significant correlation between GI and volume of GCF r (0.261) p-value (0.198) . Moreover, there was weak positive none significant correlation between GI and the level of **interleukin -1 (IL-1)** in GCF r (0.132) ,p-value (0.520) .While there was negative none significant correlation between GI and the level of IL6 in GCF r (-0.148) p-value (0.471).
IV.2. 3 Correlation between BOP% and other variables
There was negative none significant correlation between BOP% and the level of IL6 in GCF r (-0.297) p-value (0.141). Also, There was negative none significant correlation between BOP% and concentration of interleukin -1 (IL-1) in GCF r (-0.140) , p-value(0.496). while there was weak positive none significant correlation between BOP% and volume of GCF r (0.289), p-value (0.152).
IV.2. 4 Correlation between volume of GCF and other variables
There was negative high significant correlation between volume of GCF and concentration IL-6 r (-0.509) ,p-value(0.008). While there was weak positive none significant correlation between volume of GCF and concentration interleukin -1 (IL-1) in GCF r (0.066) ,p- value(0.748).
IV.2. 5 Correlation between level of Il-B and IL- 6
There was weak positive non- significant correlation between level of interleukin -1 (IL-1) and IL-6 r (0.056) ,p-value(0.786) in GCF .
I**V.3 Correlation between variables in chronic periodontitis group** Table (4) illustrates the correlation among variables in chronic periodontitis group
IV.3 .1Correlation between PLI and other variables
There was weak positive non-significant correlation PLI and the level of IL6 in GCF r (0.253), p-value (0.213).In addition ,there was weak positive none significant correlation between PLI and the level of interleukin -1 (IL-1) in GCF r (0.171), p-value (0.405).There was weak positive non-significant correlation between PLI and volume of GCF r (0.394)) ,p-value (0.046) . Also, there was positive weak none significant correlation between PLI and BOP% r (0.333) ,p-value (0.097) . In addition, There was weak positive non-significant correlation between PLI and CAL r (0.350) ,p-value (0.080).Furthermore, there was weak positive non- significant correlation between PLI and PD r (0.278), p-value (0.170).While There was moderate positive high significant correlation between PLI and GI r (0.662) , p-value (0.000).
IV.3 .2 Correlation between GI and   other variables
The correlation between GI and BOP% is strong positive high significant r (0.731) p-value (0.000). The correlation between GI and volume of GCF moderate positive high significant r (0.490) p- value (0.011).
There was weak positive non- significant correlation between GI and CAL r (0.351) p-value (0.079).Also There was weak positive non- significant correlation between GI and PD r (0.269) p-value (0.185).in addition, there was weak positive none significant correlation between GI and the level of IL6 in GCF r(0.371) p-value (0.062).Moreover , there was weak positive significant correlation between GI and the level of interleukin -1 (IL-1) in GCF r (0.395),p-value (0.046).
IV.3 .3Correlation between BOP and other variables
There was moderate positive high significant correlation between BOP% and the level of IL6 in GCF , r (0.608) p-value (0.001). In addition ,there was moderate positive high significant correlation between BOP and concentration of interleukin -1 (IL-1   )in GCF r (0.613) p-value(0.001).While There was weak positive high significant correlation between BOP and volume of GCF r (0.414) ,p-value (0.036) . While There was weak positive non- significant correlation between BOP and PD r (0.261) ,p-value (0.197). Moreover, there was weak positive non- significant correlation between BOP and CAL r (0.384),p-value (0.053).
IV.3 .4Correlation between CAL and other variables
There was strong positive high significant correlation between CAL and PD r (0.927), p-value (0.000) .There was moderate positive high significant correlation between CAL and the level of IL6 in GCF r (0.657), p-value (0.000).While there was weak positive none significant correlation between CAL and GCF volume (0.387), p-value (0.051).Also, there was weak positive none significant correlation between The CAL and concentration of interleukin -1 (IL-1) in GCF r (0.312), p-value(0.120) .

IV.3 .5Correlation between PPD and other variables

There was weak positive non- significant correlation between PD and concentration of interleukin -1 (IL-1) in GCF r(0.266),p-value(0.188).In addition, There was weak positive none significant correlation between PD and GCF volume(0.372) p-value (0.061).While There was moderate positive high significant correlation between PD and the level of IL6 in GCF r (0.576), p-value (0.002).

IV.3 .6Correlation GCF volume and other variables

There was moderate positive high significant correlation between GCF volume and the level of IL6 in GCF r (0.454)) p-value (0.020).In addition, There was moderate positive high significant correlation between GCF volume and concentration of interleukin -1 (IL-1) in GCF r (0.530), p-value(0.005).

IV.3 .7Correlation between interleukin -1 (IL-1 and the level of IL6 in GCF

There was moderate positive high significant correlation between the concentration of interleukin -1 (IL-1)and the level of IL6 in GCF r (0.556), p-value(0.003).

Figure (1): Method of GCF collection.

Figure (2) Periotron 6000(Harco 6000, USA),

Table (1) descriptive statistics and groups' difference

Variables	Groups	Descriptive statistics				Group's difference	
		N	Mean	S.D.	S.E.	t-test	p-value
PLI	Control	26	0.27	0.09	0.02	-8.338	0.000 (HS)
	Study	26	0.93	0.39	0.08		
GI	Control	26	0.28	0.10	0.02	-9.248	0.000 (HS)
	Study	26	1.15	0.47	0.09		
BOP%	Control	26	1.44	1.91	0.37	-7.307	0.000 (HS)
	Study	26	42.09	28.30	5.55		
GCF volume	Control	26	33.58	10.66	2.09	-8.590	0.000 (HS)
	Study	26	119.62	49.94	9.79		
IL-1B	Control	26	49.04	16.73	3.28	-14.842	0.000 (HS)
	Study	26	208.72	52.25	10.25		
IL-6	Control	26	3.13	1.71	0.34	-9.836	0.000 (HS)
	Study	26	9.76	2.98	0.58		

Table (2) Descriptive statistics of pocket depth and clinical attachment loss in Chronic periodontitis group

Variables	N	Mean	S.D.	S.E.
CAL	26	3.46	1.51	0.30
PPD	26	5.74	1.47	0.29

Table (3) correlation among variables in control group

Variables		IL-6	IL-1B	GCF volume	BOP%	GI
PLI	r	0.014	0.253	0.290	0.253	0.487
	p-value	0.947	0.212	0.151	0.213	0.012
GI	r	-0.148	0.132	0.261	0.327	
	p-value	0.471	0.520	0.198	0.103	
BOP%	r	-0.297	-0.140	0.289		
	p-value	0.141	0.496	0.152		
GCF volume	r	-0.509	0.066			
	p-value	0.008	0.748			
IL-1B	r	0.056				
	p-value	0.786				

Table (4) Correlation) among variables in C.P. group

Variables		IL-6	IL-1B	GCF volume	PPD	CAL	BOP%	GI
PLI	r	0.253	0.171	0.394	0.278	0.350	0.333	0.662
	p-value	0.213	0.405	0.046	0.170	0.080	0.097	0.000
GI	r	0.371	0.395	0.490	0.269	0.351	0.731	
	p-value	0.062	0.046	0.011	0.185	0.079	0.000	
BOP%	r	0.608	0.613	0.414	0.261	0.384		
	p-value	0.001	0.001	0.036	0.197	0.053		
CAL	r	0.657	0.312	0.387	0.927			
	p-value	0.000	0.120	0.051	0.000			
PPD	r	0.576	0.266	0.372				
	p-value	0.002	0.188	0.061				
GCF volume	r	0.454	0.530					
	p-value	0.020	0.005					
IL-1B	r	0.556						
	p-value	0.003						

V. Discussion

Periodontitis is an inflammatory disease leads to local elevation in levels of pro-inflammatory cytokine which plays a vital role in the process of inflammation associated with the destruction of the periodontium. (40; 41) found that immune response differs extremely among subjects .

Gingival crevicular fluid is a powerful vehicle which contains various cellular and biochemical arrays for observation tissue and cell products and permits a degree of non-invasive accessibility to the periodontium. Evaluation of the markers in GCF is regard as a useful manner to determine a person's risk for periodontal disease(42) .The present study revealed that there was high significant differences in the volume of GCF between chronic periodontitis and control groups .This result was in consistence with studies of (43 ; 44) .These differences in the volume of GCF according to the disease state may indicate the variability of occasional nature of periodontal disease progression, the different stages of inflammation, severity of disease, shifts in host-bacterial interactions, or the presence of definite putative periodontal pathogenic Bacteria.

Certain cytokines have been proposed as potentially useful diagnostic or prognostic markers of periodontal destruction(45) .Cytokines are considered to play a vital role in the process of inflammation(46).IL-1 and IL-6are locally produced with in the diseased tissues of periodontium and move by GCF into the periodontal pocket(47). IL-1 is a cell immune response mediator released as an outcome of bacterial components for example , lipopolysaccharides interacting with toll-like receptors. This cytokine increases the neutrophils recruitment and the expression of adhesion molecules as well as causing vascular modification. When it produced constantly, it can result in destruction of periodontal tissue (48).

Regarding IL-1 Concentration in GCF the results of the present study have shown that there was highly significant difference in the concentration of crevicular IL-1 between the chronic periodontitis and control groups. These findings were in consistent with results of many studies (12; 49; 5; 50 ;51;52) ; 44; 53 ; 54) they demonstrated that the concentration of IL-1 in GCF was higher in patients with periodontitis than in individuals with clinically healthy sites of periodontium. In addition,(55)reported that the total amounts of IL-1 in patients with severe periodontitis was higher than in patients with mild periodontitis and healthy subjects., These results revealed that the intensity, period and dissolution of inflammation depend on changing the equilibrium between the activities of pro-inflammatory and anti-inflammatory cytokines during inflammation of periodontal tissue (57;58). .On contrast to the present study (60) showed that lower concentrations of IL-1 at diseased sites in comparison with healthy sites in both smokers and non-smokers. It was reported that in GCF, the range of IL-1 concentrations is often quite changeable(59; 60; 61).In many studies variability in cytokine of GCF may reflect the complicated multifactorial nature of the disease and variations in sampling methods and assays used for analysis of GCF(62) .In addition, collection time and inter-individual differences may have a considerable effect on the results for studies of cytokines in GCF .Moreover, the wide range in the levels of IL-1 may be attributed to the differences in accumulation of plaque and consequential inflammation(63) .Furthermore, to subject variation in immunological response (64).

In terms of concentration of interleukin 6 (IL-6)(pg./µl) in GCF, this study demonstrated that there was high significant difference between CP and control group .This finding was in agreement with those of several studies that reported significantly higher level of interleukin 6 (IL-6)in individual with periodontitis sites are than that of healthy periodontal sites (65 ; 66;67 ; 68; 69;70;71).On contrast (72) reported that there was weak correlation between quantities of IL-6 in GCF and periodontal tissue inflammation and destruction .in addition, The concentrations of IL-6 w a s significantly higher in healthy than diseased sites, while after periodontal therapy it elevated significantly. This results could be as a result of decrease of GCF volume following successful therapy.It has been suggested that in GCF the total amount of cytokine might be more representative of the disease condition as compared to its concentration(73).

GCF volumes are very different irrespective of inflammatory condition. Therefore ,it was reported that the total marker activity per Thirty Second GCF sample rather than the concentration of the marker might supply a better correlation with health or disease condition(74) .

IL-6 has direct stimulatory influences on bone resorption (75) ,although this is controversial l results in the studies, new researches with larger samples are essential to record more perfectly the expression of cytokines in process of periodontal disease .In addition ,detection methods of cytokines can also be in charge of for these variations.

VI. Conclusion

There was high significant difference between chronic periodontitis (CP) and control group in clinical parameters[Plaque index(PLI) ,Gingival Index(GI) , Bleeding on Probing (BOP), Probing Pocket Depth (PPD) and clinical attachment loss (CAL)]. In addition ,there was high significant difference between CP and control group in concentration of interleukin 1 (IL-1) and interleukin 6 (IL-6) (pg. /µl) in GCF. Local production of IL- and (IL-6) in GCF can be consider as monitor marker give information about periodontal status and elevated levels of crevicular IL-1 and IL-6 may be a valuable tool in diagnostic potentials for the early detection of periodontal disease.

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