Detection of Granulocyte Chemotactic Protein 2 in Serum of Periodontitis Patients

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ABSTRACT

Background: Chronic periodontitis defined as "an infectious inflammatory disease within supporting tissues of the teeth, progressive attachment loss and bone loss". Aggressive periodontitis is rare which in most cases manifest themselves clinically during youth. It characterized by rapid rate of disease progression .Pro-inflammatory chemokines organized inflammatory responses. Granulocyte chemotactic protein 2 is involved in neutrophil gathering and movement. The purpose of the study is to detect serum of Granulocyte Chemotactic Protein 2 and correlate to periodontal condition in patients with chronic periodontitis, Aggressive periodontitis and Healthy Control subjects and measurement the count of neutrophils for the studied groups.

Subjects and methods: Eighty four male and female were enrolled in this study .They were divided into three groups (18) patients with Aggressive periodontitis with age range (20-45) years, (33) chronic periodontitis patients and (33) Healthy control with an age range (30-50). Clinical periodontal parameters were recorded for each group. The concentration of granulocyte chemotactic protein- 2 in serum was quantified by a high-sensitivity enzyme linked immunosorbent assay. Blood neutrophils count were detect for five subjects from each group using light microscope

Result: ANOVA analysis revealed high significant differences in Granulocyte chemotactic protein 2 means between aggressive, chronic and controls. Neutrophils count in aggressive periodontitis is higher than chronic and controls .No significant difference in neutrophils count between aggressive and chronic periodontitis, while significant difference when correlate them with controls

Conclusion The concentration of granulocyte chemotactic protein 2 increased with the increase in severity of periodontitis. Higher neutrophils count was found in aggressive periodontitis than chronic and controls. As higher granulocyte chemotactic protein 2 that chemoattract more neutrophils recruitment to the site of inflammation

Keywords: Granulocyte chemotactic protein 2, aggressive periodontitis, chronic periodontitis, neutrophils. **(J Bagh Coll Dentistry 2016; 28(4):122-127)**

INTRODUCTION

Periodontitis can be defined as a range of clinical entities that are characterized by immunological destruction of the supporting tooth structures in response to chronic challenge by specific bacteria in sub gingival biofilm [1].

Periodontitis presents in two forms Chronic and Aggressive, with the possibility for both to involve a localized area or generalized involvement. This form of periodontal disease undergo defect in their immune response to dental biofilms .One of these immune defects is related to activity of neutrophils which is form the main component of human innate immune system first line defense that kill pathogens and lead to tissue healing by promoting inflammatory resolution [2].

Chemokines are produced in response to bacterial components. Chemokines are a class of chemotactic cytokines that stimulate recruitment of relatively specific leukocyte subset [3]. Granulocyte chemotactic protein 2 (GCP-2) of 6KDa (75 amino acids) is a chemokine CXC as a neutrophil chemoattractant. It produced by stimulated human osteosarcoma cells (MG-63) [4].

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MATERIALS AND METHODS

Sample selection

The subjects enrolled in the present study composed of (84) subjects. They were divided into three main groups (33) patients have CP, (18) Ag P, and (33) subject wit clinically healthy periodontium as control. The age ranged from (30-50) years for chronic and control and (20-45) years for aggressive group. All clinical parameters PI [5], GI [6], BOP [7], PPD [8] and CAL were recorded for each group.

Under a strict aseptic condition a 5ml venous blood was withdrawn from each subject. Blood sample was collected into EDTA tubes .After centrifugation for 10 minutes at 4000 rpm to separate serum from blood and collected in eppendrof and kept in the deep freeze at 20 ˚C till used. Results were calculated using the standard curves created in each assay. A concentration of the granulocyte chemotactic protein 2 was corrected for serum defined as (pg/ml). For measuring neutrophils count a 3ml of venous blood was withdrawn from 5 subjects of each group. Blood sample was collected into EDTA tubes. The count done (Cells/ µl.) using light microscope.

Assay procedure/ Human serum GCP2 ELISA Kit, China

Equilibrate all materials and prepared reagents to room temperature (18 - 25°C) prior to use. It is recommended to assay all standards, controls and samples in duplicate

1. Add 100 µL of each standard and sample into appropriate wells. Cover well and incubate for 2.5 hours at room temperature or over night at 4°C with gentle shaking .
2. Discard the solution and wash 4 times with 1X Wash solution. By filling each well wash with Wash Buffer solution (300 ml) using a multi-channel pipette or autowasher.
3. Add 100 µL of 1X prepared biotinylated antibody to each well. Incubate for hour at room temperature with gentle shaking
4. Discard the solution. Repeat the wash as in step2
5. Add 100 µL of TMB One-Step Substrate Reagent to each well. Incubate for 30 minute at room temperature in the dark with gentle shaking.
6. Add 50 µL of Stop Solution to each well. Read at 450 nm immediately (Figure 1).

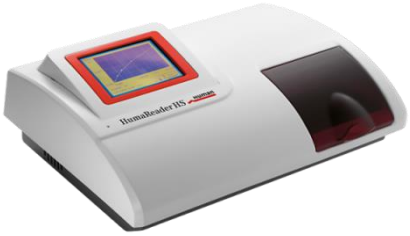

Figure1: ELISA (Human-Germany)

Procedure of neutrophils count

After 3ml blood collection into EDTA tubes , put blood into slide and make a smear .Let it to dry for 10 minutes then Lishmans stain add to it and let to dry for (1.5) minute, after that diluted with distilled water for 6 minutes then leave it for study under light microscope.

Statistical Analyses

All patients' data entered using computerized statistical software; Statistical Package for Social Sciences (SPSS) version 17 was used.

A- Descriptive statistics presented as (mean ± standard deviation),

B- Frequencies and percentages. Multiple contingency tables conducted and appropriate statistical tests performed,

C- Chi Square test was used to compare frequencies and percentage between any two groups

D- Fishers exact test was used if more than 20% expected variables were less than 5.

P value of more than 0.05 was regarded as statistically insignificant as follows:

$p>0.05$ NS Non-significant

$0.05\geq p>0.01$ * Significant

$0.01\geq p>0.001$ ** highly significant

RESULTS

Descriptive Analysis

The result of this study based on the analysis for the sample of (84) male and female patients, (18) with Aggressive periodontitis, (33) with chronic periodontitis and (33) subjects with clinically healthy periodontium as control .The age of the patients ranged between (20-40) years for Aggressive periodontitis, (30-50) years for CP group and control group. The mean age of the participants was (37±8) years. The percent of participant male was 53.6 % and 46.4 % female. As shown in table (1). Aggressive periodontitis patients were significantly associated with younger ages and chronic periodontitis patients were significantly associated with older age ($p<0.001$). No significant differences were observed between periodontitis patients and controls regarding their gender ($p=0.6$), table (1).

Table 1: Demographic characteristics and distribution of the study participants

Variable	Aggressive		Chronic		Control		Statistical Test	p	Sig.
Age mean	28.8±7.3		41.9±7.3		35.3±5		F=24.2 df=2	<0.001	HS
Age mean ± SD (37±8 years) for all groups									
Gender									
Variable	No.				%				
Female	45				53.6				
Male	39				46.4				
Total	84				100.0				
Gender	Aggressive		Chronic		Control		Statistical test	P	Sig.
	No.	%	No.	%	No.	%			
Male	8	17.8	19	42.2	18	40.0	χ^2=0.8	0.6	NS
Female	10	25.6	14	35.9	15	38.5			

*Fishers exact test.

Clinical periodontal parameters

Plaque index (PLI)

The mean of PLI in chronic periodontitis group **(Mean±SD)** was (1.3±0.3), while in aggressive periodontitis (**Mean±SD)** was (1±0.18). Statistically a highly significant difference appeared between these two means. Plaque index for controls was (0.5± 0.3). As shown in table (2).

Gingival index (GI)

The mean values of GI were higher in chronic periodontitis group (1.37±0.48) than Aggressive periodontitis (1.25±0.53). This difference in GI for CP and AgP statistically non- significant .GI for controls was (0.24± 0.21) .As shown in table (2).

Probing pocket depth (PPD)

The mean values of PPD were higher in Aggressive periodontitis group (4.59±0.52) than chronic periodontitis group (4.38±0.48). As shown in table (2).Statistically this difference is of anon-significant.

Clinical attachment level (CAL)

In table (2) reveal that the mean values of CAL was higher in Aggressive periodontitis group (3.3±0.87) than chronic periodontitis group (3.06±0.97).This difference statistically non-significant value.

Table 2: Distribution and statistical difference of clinical parameters in study groups

Groups (Mean±SD)	Pl. I (Mean±SD)	GI (Mean±SD)	PPD (Mean±SD)	Min. pocket	Max. pocket	CAL (Mean±SD)
Aggressive	1±0.18	1.25±0.53	4.59±0.52	4	8	3.3± 0.87
Chronic	1.3±0.3	1.37±0.48	4.38±0.48			3.06±0.97
Control	0.5±0.3	0.42±0.21				
Statistical test	t-test=3.5 P=**0.001**	t-test =0.8 P=0.4	t-test=1.4 P=0.1			t-test=0.9 P=0.3

Bleeding on probing (BOP)

In Descriptive statistics for BOP the number of sites examined for aggressive periodontitis (1764), in chronic periodontitis group were (3292). The sites that bleed were described as score 1 while non bleeding sites were described as score 0 as shown in table (3).

For both groups the % of bleeding sites were much lower than the non-bleeding sites. It was (13.435%) for aggressive periodontitis and (11.817%) for chronic periodontitis. Chi square test revealed a non-significant difference between them.

Table 3: The statistical difference in the percentage of sites BOP between aggressive and chronic periodontitis

Total site number	Percentages	BOP		Chi	p-value	Sig
		Score 0	Score 1			
Aggressive periodontitis No.=1764	**No.**	1527	237	0.9	0.7	NS
	%	86.565	13.435			
Chronic periodontitis No.=3292	**No.**	2903	389			
	%	88.183	11.817			

Immunological parameter

Descriptive and Statistical analysis of GCP2 among all studied groups

The concentration of GCP2 (pg./ml) higher in serum of Aggressive periodontitis group(919.14±217.3) than in chronic periodontitis group (571.9±172.6) and in control group (419.5±249.9).

ANOVA analysis revealed high significant difference in GCP2 means between aggressive, chronic periodontitis and controls (Table 4).

Table 4: Inter group comparison of GCP2 conc. (pg/ml) among all studied groups

Groups	GCP2 (Mean±SD)	df	F	P	Sig.
Aggressive	919.14±217.3	2	47.5	**<0.001**	HS
Chronic	571.9±172.6				
Control	419.5±249.9				

Difference in GCP2 conc. according to gender

Among aggressive and chronic periodontitis patients, there was no significant difference in GCP2 means between males and females (p>0.05), on other hand, significant difference in GCP2 means between males and females among healthy controls, GCP2 mean was higher among female controls (p=0.04) (Table 5).

Table 5: Intra group differences of GCP2 conc. according to gender for all studied groups

Groups	Male (Mean±SD)	Female (Mean±SD)	Male (Mean±SD)	Female (Mean±SD)	Male (Mean±SD)	Female (Mean±SD)
GCP2	900.6±283.3	915.9±163.4	586±123.6	552.9±226.8	374.9±144.7	473.1±117.1
Statistical test	t-test=0.1 P=0.8		t-test=0.6 P=0.5		t-test=2.1 P=**0.04**	

Inter groups Statistical Difference in GCP2 in both male and female

ANOVA analysis revealed high significant differences in GCP2 means between aggressive, chronic periodontitis and controls in both males and females (p<0.001) (Table 6).

Table 6: Inter-groups statistical difference of GCP2 conc. According to gender for all studied groups

Groups	Male (Mean±SD)	Female (Mean±SD)
Aggressive	900.6±288.3	915.9±163.4
Chronic	586±123.6	552.9±226.8
Control	374.9±144.7	473.1±117.1
ANOVA	d.f=2, F=27.4, P=<**0.001**	d.f=2, F=20.6, P=<**0.001**

Correlation between clinical parameters and immunological parameter

Correlation between clinical parameters of aggressive periodontitis and GCP2 conc

A significant moderate negative correlation was observed among aggressive periodontitis patients between mean plaque index and GCP2 mean (p=0.01). There was weak positive significant correlation between probing pocket depth, clinical attachment loss and bleeding on probing means with GCP2 mean (p<0.05). No significant correlation was observed between gingival index and GCP2 means (p=0.4) (Table 7).

Table 7: Pearson correlation between GCP2 conc. and clinical parameters for aggressive periodontitis patients

Variable	GCP2			Sig.
	r	d.f	P	
Pl. I	-0.6	15	**0.01**	S
GI	0.1	15	0.4	NS
PPD	0.3	15	**0.04**	S
CAL	0.3	15	**0.01**	S
BOP	0.2	15	**0.02**	S

Correlation between clinical parameters of chronic periodontitis and GCP2conc

A significant weak positive correlation was observed among chronic periodontitis patients between mean GI and GCP2 mean (p=0.05). No significant correlation was observed between plaque index, probing pocket depth, clinical attachment loss and bleeding on probing means with GCP2 means (p=>0.05), (Table 8).

Table 8: Pearson's correlation between GCP2 and clinical parameters for chronic periodontitis patients.

Variables	r	d.f	P	Sig.
Pl. I	-0.02	30	0.8	NS
GI	0.3	30	**0.05**	S
PPD	-0.07	30	0.6	NS
CAL	0.02	30	0.8	NS
BOP	0.03	30	0.6	NS

Correlation between clinical parameters of controls and GCP2 conc.

No significant correlation was observed among healthy controls between plaque index and gingival index means with GCP2 means (p=>0.05) (Table 9).

Table 9: Pearson correlation between GCP2 conc. and clinical parameters for healthy controls

Variables	R	d.f	P	Sig.
Pl. I	0.03	30	0.8	NS
GI	-0.07	30	0.7	NS

Inter group Difference in neutrophils count (Cells/μl) between aggressive periodontitis and controls

Statistically a significant difference between the means of neutrophil count between AgP and CP groups (p=0.61). Although the higher mean was within AgP group. On the other hand statistically a significant difference between the means of neutrophil count of AgP and Controls groups and between CP and Controls (Table 10).

Table 10: Mean of Neutrophils count (Cells/μl) among studied groups

Groups	Neutrophils count (Mean± SD)
Aggressive	3876.0 ± 454.18
Chronic	3724.0 ± 410.95
Control	2926.0 ± 374.005

Inter group Difference in neutrophils count (Cells/μl) between aggressive periodontitis and controls

Statistically a significant difference between the means of neutrophil count between AgP and CP groups (p=0.61). Although the higher mean was within AgP group. On the other hand statistically a significant difference between the means of neutrophil count of AgP and Controls groups and between CP and Controls (Table 11).

Table 11: Distribution and statistical difference in neutrophils count (Cells/μl) between studied groups

Groups	Neutrophils count (Mean ±SD)	Statistical test	Sig.
Aggressive	3876.0 ± 454.18	t-test=0.55 p=0.61	NS
Chronic	3724.0 ± 410.95		
Aggressive	3876.0 ± 454.18	t-test=3.13 p=0.03	S
Control	2926± 374.005		
Chronic	3724.0 ± 410.95	t-test=4.07 p=0.01	S
Control	2926± 374.005		

DISCUSSION

The aim of this study is to evaluate the serum level of GCP2 in patients with AgP, CP and healthy controls. The study is the first of its kind and there are no previous studies comparing the serum level of GCP2 between the three mentioned groups, therefore comparing the results not possible.

The most exposed to AgP were young age and that agreed to the [9] and as in past classification of periodontitis [10,11],although elimination the age criteria is possibly one of the most important innovations of AAP 1999 consensus classification of periodontal disease published by Armitage [12]. Also in our study CP affect people of all ages and with age increase and this agreed with Albandar and Rams [13].

Chronic periodontitis group comprised of significantly elder patients compared to AgP and healthy controls groups and that agreed with Cifcibasi et al. [14]. Genetic or systemic factor are associated with CP, while the predisposing factor for AgP is the families history of periodontal disease. According to Fourel [15], one of the constant parameters in EOP currently classified as AgP is the existence of a familial factor .

PI scores were significantly higher in CP than AgP and healthy controls and that agreed with Cifcibasi et al. [14] and disagreed with Anisehnaderan et al. [16].

No significant difference in mean of GI, PPD, CAL between chronic and aggressive periodontitis and that agreed with Cifcibasi et al. [14] and disagreed with Benoist et al. [17].

The level of serum GCP2 is higher in periodontitis patients than controls as in result of Kebsckull et al. [18] who showed a high expression of GCP2 in disease gingival tissue than healthy gingival tissue and the level of GCP2 in the study of Kebsckull et al. [18] correlate positively with PPD and not with CAL that reflects current periodontal inflammatory status and not history of periodontitis. While the GCP2 level in the present study correlate positively even it is a weak correlation with PPD and CAL

A significant difference was found in neutrophils count between periodontal diseases and controls. Low count in controls and high count in diseases and that is agreed with Bender et al. [19].

High count of neutrophils in aggressive periodontitis agreed with Buchmann et al. [20] and disagreed with Genco [21]. Also elevated neutrophils count in chronic periodontitis agreed with Hidalgo et al. [22]. As high GCP2 in aggressive periodontitis that increased chemotactant of neutrophils to the inflamed sites that increase the destruction of tissues in AgP and that is agreed with Kantarci and Van Dyke [23].

REFERENCES

1. Darveau RP. Periodontitis: a polymicrobial disruption of host homeostasis. Nat Rev Microbiol 2010; 8(7): 481-90.

2. Scott DA, Krauss J. Nutrophils in periodontal inflammation. Front oral Biol 2012; 15: 56-83
3. Oppenheim J, Zachariae C, Mukaida N, Matsushima K. Properties of the novel proinflammatory supergene ‘‘intercrine’’ cytokine family. Ann Rev Immunol 1991; 9: 617 – 48.
4. Proost P, De Wolf-Peeters C, Conings R, et al. Identification of a novel granulocyte chemotactic protein (GCP-2) from human tumor cells. In vitro and in vivo comparison with natural forms of GRO, IP-10, and IL-8 . J Immunol 1993; 150:1000-10.
5. Silness P, Löe H. Periodontal disease in pregnancy. Acta Odontol Scand 1964; 22:121.
6. Löe H. The gingival index, the plaque index and the retention index system. J Periodontal 1967; 38(6): 610-6
7. Newman MG, Takei H, Klokkevold PR, Carranza FA. Carranza’s Clinical Periodontology. 11th ed. St. Louis: Elsevier, Saunders; 2012.
8. Lang NP, Bartold PM, Cullinam M, et al. International classification workshop. Consensus report: chronic periodontitis. Annals of Periodontol 1999; 4: 53
9. Alpagot T, Wolff LF, Smith QT, Tran SD. Risk indicators for periodontal disease in a racially diverse urban population. J Clin periodontal 1996; 23: 982-8
10. Ranney RR. Classification of periodontal diseases. Periodontal 2000 1993; 2: 13-25
11. Suzuki JB, Charon JA. Current classification of periodontal diseases. J Parodontal 1989; 8: 31-51.
12. Armitage GC. Development of a classification system for periodontal diseases and conditions. Ann Periodontol 1999; 4: 1–6.
13. Albandar JM, Rams TE. Global epidemiology of periodontal diseases: an overview. Periodontol 2000 2002; 29: 7-10.
14. Cifcibasi E, Ciblak M, Kiran B, Badur S, Firatli E, et al. The role of activated cytotoxic T cell in Etiopathogesis of periodontal Disease: Does It harm or Does It heal? 2015
15. Fourel J. Periodontitis: a periodontal syndrome. J Periodontal 1972; 43: 240-255
16. Anisehnaderan M, Ebrahimpoor M, Sadeghipoor M. Association between psychological stress and stimulation of inflammatory responses in periodontal disease. J Dent 2013; 10(1): 103-11
17. Benoist HM, Seck-Diallo A, Diouf A, Yabbre S, Sembene M, Diallo PD. Profile of chronic and aggressive periodontitis among Senegalese. J Periodont Implant Sci 2011; 41(6): 279-84.
18. Kebsckull M, Demmer R, Behle JH, Pollreisz A, Heidemann J, et al. Granulocyte Chemotactic Protein 2 (GCP-2/CXCL6) complements interlukin 8 in periodontal disease. J Periodontal Res 2009; 44: 465-71.
19. Bender JS, Thang H, Glogauer M. Novel rinse assay for the quantification of oral neutrophils and the monitoring of chronic periodontal disease. J Periodontal Res 2006; 41: 214-20
20. Buchmann R, Hasilik A,Van Dyke TE, Lang DE. Amplified crevicular leukocyte activity in aggressive periodontal disease. J Dent Res 2002; 81: 716-21
21. Genco RJ. Current view of risk factors for periodontal diseases. J Periodontol 1996; 67:1041–9
22. Hidalgo MM, Avila-Campos MJ, Trevisan W, Jr, Mocelin TT, Itano EN. Neutrophil chemotaxis and serum factor modulation in Brazilian periodontitis patients. Arch Med Res. 1997; 28: 531–5.
23. Kantarci A, Van Dyke TE. Lipoxin signaling in neutrophils and their role in periodontal disease. Prostaglandins Leukot Essent Fatty Acids 2005; 73: 289-99.