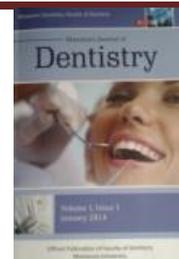




## Evaluation of Pentraxin-3 Level in Gingival Crevicular Fluid in Smoker and Non-smoker Patients with Chronic Gingivitis and with Chronic Periodontitis



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### Abstract:

**Objectives:** The aim of this study was to evaluate PTX3 level in gingival crevicular fluid (GCF) in smoker and non-smoker individuals complaining from chronic gingivitis and chronic periodontitis.

**Methods:** Sixty male participants were involved in the study. Participants were divided into two groups based on gingival index (GI), probing pocket depth (PPD), clinical attachment loss (CAL), and smoking habit: study group (smoker gingivitis sub-group; n=10) (SG), (smoker periodontitis sub-group; n=10) (SP), (non-smoker gingivitis sub-group; n=10) (NSG), (non-smoker periodontitis sub-group; n=10) (NSP), and control group (positive control group; n=10) (+veC), (negative control group; n=10) (-veC). GCF samples collected from each subject were quantified for PTX3 levels using an enzyme-linked immunosorbent assay.

**Results:** The mean PTX3 concentrations increased in GCF from healthy to gingivitis to periodontitis. Smokers had lower PTX3 concentrations increased in GCF than non-smokers. PTX3 concentrations also correlated positively with periodontal parameters.

**Conclusions:** PTX3 level in GCF was significantly elevated in periodontitis patients than gingivitis patients and healthy subjects. GCF PTX3 concentrations correlated positively with periodontal parameters. Smoking has a suppressive effect on PTX3 level in GCF of smokers.

**Keywords:** Smoking, PTX3, GCF.

### Introduction

Periodontal diseases are initiated by Gram-negative tooth-associated microbial biofilms that elicit a host response with resultant osseous and soft tissue destruction [1]. Numerous studies have indicated that smoking is a significant risk factor for the development of periodontal disease [2]. The composition of tobacco smoke is a complex mixture of between 2,000 and 3,000 toxic substances [3]. Chronic inhalation of cigarette smoke alters a wide range of immunological functions, including innate and adaptive immune responses [4]. Many of researches indicated that smoking may interfere with several reparative and destructive factors such as the function of inflammatory cells and production of immune mediators [5-7].

With current periodontal diagnostic tools it is difficult to recognize susceptible individuals or sites. We have no means of predicting when gingivitis is developing into periodontitis or when periodontitis is in a progressive state with increases in PPD and further CAL. Clinical parameters such as PPD and CAL, as well as radiological findings, indicates the disease history but not necessarily the status at that particular moment. Bleeding on probing (BOP) has been regarded as a useful negative predictor of gingival health in most cases [8]. However, the finding of reduced bleeding sites in smokers has confounded this relationship [9].

Acute-phase proteins, cytokines, and prostaglandins are inflammatory mediators produced as a part of host response that contribute to tissue destruction [1,10]. Data show that acute-phase proteins, plasma proteins, not only appear in acute inflammation, but also in longstanding, chronic conditions [11]. Acute-phase proteins are generally increased following a microbial infection. It is, therefore,

possible that acute-phase proteins are sensitive markers to evaluate inflammatory status of various microbial infections [12]. Pentraxins (PTX3), a superfamily of acute-phase proteins, are an essential component of the humoral arm of innate immunity [13]. PTX3 was the first long PTX described as an IL-1 $\beta$  inducible gene in endothelial cells [14]. It is produced by a variety of cells, mostly by cells abundant in periodontal tissues [15]. Recent clinical studies were published suggesting that PTX3 in GCF is considered a diagnostic marker of the periodontal disease inflammatory activity [1,16]. So, this study was performed to evaluate PTX3 level in gingival crevicular fluid in smoker and non-smoker individuals complaining from chronic gingivitis and chronic periodontitis.

### Materials and methods

The present study was carried out on sixty male patients, who were selected from the department of Oral Medicine and Periodontology, Faculty of Dentistry, Mansoura University. The age of patients was ranged from 35-60 years and half of them were smokers. Participants were divided into two groups based on gingival index, probing depth, clinical attachment level, and smoking habit: study group (smoker gingivitis sub-group; n=10) (SG), (smoker periodontitis sub-group; n=10) (SP), (non-smoker gingivitis sub-group; n=10) (NSG), (non-smoker periodontitis sub-group; n=10) (NSP), and control group (positive control group; n=10) (+veC), (negative control group; n=10) (-veC).

The selected patients were free from any autoimmune or systemic diseases, and receiving no medications like antibiotic and anti-inflammatory drugs which may affect microbial flora, immune system, or the inflammatory response for at least the last 3 months. Furthering, none of

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them had periodontal treatment in the last six months. Only one site per participant was selected in diseased groups, whereas, in control groups; two sites were pooled to ensure the collection of an adequate amount of GCF. In patients with gingivitis, the site with the highest clinical signs of inflammation (i.e., redness, bleeding on probing, and edema) was selected for sampling. In patients with chronic periodontitis, site that showed the highest CAL and signs of inflammation was selected for sampling [1]. GCF was collected by placing a sterile endodontic paper point size 25 into the sulcus until mild resistance, and left in place for 30 seconds according to the recommendations of Lamster et al., (1985) [17]. Paper point was removed and placed in a preweighed Eppendorf tube. The GCF weight was measured by highly sensitive scale then tubes were stored at -20°C. The weight of the fluid in each paper point was converted to volume by assuming that the density of GCF is 1.0 mg/mL [18]. Each strip was placed in 500 µL phosphate-buffered saline (pH = 7.4) and left overnight at 4°C in order to elute GCF. The samples were then mixed three times for 3 seconds each using a vortex. The strip debris was removed by centrifugation at 400×g for 10 minutes and the supernatant harvested. The elute was divided into aliquots to be assayed [19]. PTX-3 levels in GCF were determined using a commercial ELISA kit (Human Pentraxin 3/TSG-14 Quantikine ELISA kit, R&D Systems, Minneapolis, Minn, Catalog Number DPTX30).

### Results

GI in smoker gingivitis sub-group showed a highly significant lower mean value (mean:  $1.6 \pm 0.516$ ; range: 1-2) versus non-smoker gingivitis sub-group (mean:  $2.3 \pm 0.483$ ; range: 2-3) at  $p=0.006$ . GI in smoker periodontitis sub-group showed a very highly significant lower mean value (mean:  $1.4 \pm 0.516$ ; range: 1-2) than non-smoker periodontitis sub-group (mean:  $2.4 \pm 0.516$ ; range: 2-3) at  $p= 0.000$ . GI in smoker group showed a very highly significant lower value (mean:  $1 \pm 0.831$ ; range: 1-2) (mean:  $1.567 \pm 1.194$ ; range: 2-3) of GI than smoker group (mean:  $1.567 \pm 1.194$ ; range: 2-3) at  $p= 0.000$ .

PPD in smoker periodontitis sub-group showed significant higher mean value (mean:  $4.580 \pm 0.813$ ; range: 3.1 – 5.9) than non-smoker periodontitis sub-group mean value (mean:  $5.735 \pm 1.258$ ; range: 4.1 – 7.7) at  $p= 0.025$ . Concerning CAL, smoker periodontitis sub-group showed a highly significant higher mean value (mean:  $4.93 \pm 1.020$ ; range: 3.5 – 6.6) than non-smoker periodontitis sub-group mean value (mean:  $3.62 \pm 0.871$ ; range: 2.75 – 4.6) at  $p= 0.005$ .

PTX3 level showed a very highly significant higher volume in smoker gingivitis sub-group (mean:  $0.444 \pm 0.144$ ; range: 0.3 – 0.79) versus positive control sub-group (mean:  $0.142 \pm 0.051$ ; range: 0.07 – 0.22) at  $p= 0.000$ . Smoker periodontitis sub-group showed a very significant higher volume of PTX3 (mean:  $0.965 \pm 0.475$ ; range: 0.46 – 1.84) versus positive control sub-group (mean:  $0.142 \pm 0.051$ ; range: 0.07 – 0.22) at  $p= 0.000$ . Smoker periodontitis sub-group showed significant higher volume of PTX3 level (mean:  $0.965 \pm 0.475$ ; range: 0.46 – 1.84) versus smoker gingivitis sub-group (mean:  $0.444 \pm 0.144$ ; range: 0.3 – 0.79) at  $p= 0.027$ .

PTX3 level showed a highly significant higher volume in non-smoker gingivitis sub-group (mean:  $0.656 \pm 0.031$ ;

range: 0.4 – 0.84) versus negative control sub-group (mean:  $0.292 \pm 0.068$ ; range: 0.2 – 0.45) at  $p= 0.004$ . Non-smoker periodontitis sub-group showed a very highly significant higher volume of PTX3 level (mean:  $1.740 \pm 0.615$ ; range: 0.9 – 2.8) versus negative control sub-group (mean:  $0.292 \pm 0.068$ ; range: 0.2 – 0.45) at  $p= 0.000$ . Non-smoker periodontitis sub-group showed a highly significant higher volume of PTX3 level (mean:  $1.740 \pm 0.615$ ; range: 0.9 – 2.8) versus non-smoker gingivitis sub-group (mean:  $0.656 \pm 0.031$ ; range: 0.4 – 0.84) at  $p= 0.002$ .

PTX3 level showed a highly significant lower volume in smoker gingivitis sub-group (mean:  $0.444 \pm 0.144$ ; range: 0.3 – 0.79) versus non-smoker gingivitis sub-group (mean:  $0.656 \pm 0.031$ ; range: 0.4 – 0.84) at  $p= 0.009$ . Smoker periodontitis sub-group showed a highly significant lower volume of PTX3 level (mean:  $0.965 \pm 0.475$ ; range: 0.46 – 1.84) versus non-smoker periodontitis sub-group (mean:  $1.740 \pm 0.615$ ; range: 0.9 – 2.8) at  $p= 0.006$ . Positive control sub-group showed a very highly significant lower volume of PTX3 level (mean:  $0.142 \pm 0.051$ ; range: 0.07 – 0.22) versus negative control sub-group (mean:  $0.292 \pm 0.068$ ; range: 0.2 – 0.45) at  $p= 0.000$ . Smoker group showed significant higher volume of PTX3 level (mean:  $0.517 \pm 0.444$ ; range: 0.07 – 1.84) versus non-smoker group (mean:  $0.896 \pm 0.721$ ; range: 0.2 – 2.8) at  $p= 0.017$ .

We found that GI, PPD, and CAL in smoker gingivitis sub-group showed no correlation with the mean level of PTX3. Also, in non-smoker gingivitis sub-group GI, PPD, and CAL showed no correlation with the mean level of PTX3. Moreover, we found that smoker periodontitis sub-group showed a very higher positive correlation with GI at  $r=0.853$  and  $p=0.000$ , higher positive correlation with CAL at  $r=0.88$  and  $p=0.002$ , and positive correlation with PPD at  $r=0.661$  and  $p=0.044$  when correlated with the mean level of PTX3. In non-smoker periodontitis sub-group we noticed that, GI and PPD showed no correlation with the mean level of PTX3, but CAL showed a higher positive correlation with the mean level of PTX3 at  $r=0.73$  and  $p=0.000$ . Very strong positive correlations were found between the mean level of PTX3 and the recorded clinical parameters in study group; GI ( $r=0.65$ ;  $p=0.000$ ), PD ( $r=0.72$ ;  $p=0.000$ ), and CAL ( $r=0.73$ ;  $p=0.000$ ).

### Discussion

Advances of periodontal disease diagnostic research are moving toward methods whereby periodontal risk can be identified and quantified by objective measures such as biomarkers [20]. PTX3, a newly discovered inflammation marker, is a member of acute-phase proteins [15]. Recent clinical studies were published suggesting that PTX3 in GCF is considered a diagnostic marker of the periodontal disease inflammatory activity [1,16]. Therefore, the aim of this study was to determine the effect of smoking on the level of PTX3 in GCF in smokers and non-smokers complaining from chronic gingivitis and chronic periodontitis.

It was found that there was a significant higher mean value of GI in non-smoker gingivitis sub-group versus smoker gingivitis sub-group, non-smoker periodontitis sub-group versus smoker periodontitis sub-group, and non-smoker group versus smoker group. These results are consistent with Bajagić et al. [21] who mentioned that smokers commonly present with fibrotic gingiva, less

gingival redness and limited edema relative to disease severity. Smoking reduction of clinical signs of gingival inflammation in chronic gingivitis and chronic periodontitis illustrated as NT stimulation of the sympathetic ganglia to produce neurotransmitters including catecholamines which affect the alpha-receptors on blood vessels that in turn cause vasoconstriction [22].

Comparing PPD in smoker periodontitis sub-group and non-smoker periodontitis sub-group, there was a significant higher mean value of PPD in smoker periodontitis sub-group. These results are consistent with Mullally [23] who reported that the percentage of sites with probing depths in excess of 4mm was more than double in young smokers (15%) compared with (6%) in non-smokers. This may mean that the increased pocket depth in smokers could be due to immunosuppressive effect of smoking, excessive destruction, and repair impairment in periodontal tissues. Bergstrom [24] explained the more tissue destructive effect of smoking by its interference with vascular and inflammatory processes in periodontal tissues.

Studying the CAL of smoker and non-smoker periodontitis sub-groups, a significant higher value was found in smoker periodontitis sub-group versus non-smoker periodontitis sub-group. These results are consistent with Machtei et al. [25] who found that the extent of periodontitis as evaluated by the percentage of sites with attachment loss more than 2mm was (22%) for young adults who smoked compared with (9%) in those who did not. This may clarify that the attachment loss in chronic periodontitis patients is increased by smoking due to increased bone destruction with impaired repair mechanisms due to immune cell affection by smoking. Jelan et al. [26] demonstrated that tobacco smoking affects multiple functions of neutrophils and shift the net balance of neutrophil activities into the more destructive direction.

Concerning PTX3 level comparisons, we found that there were significant higher volumes of PTX3 in smoker gingivitis sub-group versus positive control sub-group, smoker periodontitis sub-group versus smoker gingivitis sub-group, and smoker periodontitis sub-group versus positive control sub-group. The same results were found in non-smoker sub-groups; there were significant higher volumes of PTX3 in non-smoker gingivitis sub-group versus negative control sub-group, non-smoker periodontitis sub-group versus negative control sub-group and non-smoker periodontitis sub-group versus non-smoker gingivitis sub-group. These results are in agreement with Fujita et al. [16] who demonstrated that, the GCF PTX3 level was significantly higher in periodontal lesions as compared to periodontally healthy sites in patients with chronic periodontitis.

On the contrary, Keles et al., (2012)<sup>15</sup> in their experimental periodontitis study model found that, gingival tissue PTX3 levels were not increased in experimental periodontitis model with 40-days period versus 10-days period. The present study results clarify that PTX3 level in GCF depends on the severity of periodontal disease and this could be confirmed by PTX3 active secretion by the predominant cells of periodontal disease activity.

Our study results revealed that non-smoker healthy subjects showed significant higher mean volumes of PTX3 levels versus smoker healthy subjects, non-smoker gingivitis sub-group versus smoker gingivitis sub-group, non-smoker periodontitis sub-group versus smoker periodontitis sub-group, and overall non-smoker group versus smoker group. These results are in agreement with studies which revealed that smoking is a potent inhibitor for the activity and amounts of chemokines and proinflammatory cytokines [27,28].

Inoue et al. [29] performed a study on patients with unstable angina pectoris, he reported that PTX3 levels were independent of total cholesterol, HDL cholesterol, hemoglobin A1C, smoking status, gender, or obesity. Besides, Pauwels et al. [30] reported that, in cigarette smoke model of chronic obstructive pulmonary disease, it was demonstrated that sub-acute and chronic cigarette smoke exposure significantly upregulates PTX3 expression in endothelial cells of lung veins. The present study illustrated that smoking decreases PTX3 level in GCF. Accordingly, we may be able to say that the smoking has an immunosuppressive effect.

In the present study we found that PTX3 mean levels have no correlation with GI, PPD, and CAL in both smoker gingivitis and non-smoker gingivitis sub-groups. In smoker periodontitis sub-group, there was a high positive correlation between PTX3 mean levels and GI, PPD, and CAL. But in non-smoker periodontitis sub-group there was a high positive correlation between PTX3 mean levels and CAL only and there was no correlation with GI and PPD. When clinical parameters were correlated with PTX3 mean levels in study group as a whole we found that there was a strong positive correlation with GI, PPD, and CAL.

These results are consistent Fujita et al., (2012)<sup>16</sup> who found a strong positive correlation between mean clinical parameters and GCF volume with the mean level of PTX3. Furthermore, Lakshmanan et al. [31] showed that the mean level of PTX3 levels in the gingival tissues correlated positively with clinical parameters and GCF volume in chronic periodontitis, aggressive periodontitis, and healthy subjects. The present study results demonstrated that PTX3 levels correlates positively with clinical signs of periodontal diseases. But we may need greater number of patients to confirm its positive correlation in all sub groups of the study. This positive correlation may be due to local secretion of PTX3 by neutrophils in the inflammation site. So, its concentration in GCF may be correlated with the inflammation severity.

### Conclusion

1. PTX3 level in GCF was significantly elevated in chronic gingivitis and chronic periodontitis patients when compared with healthy subjects, as well as in chronic periodontitis patients when compared with chronic gingivitis ones.
2. PTX3 correlate positively with clinical parameters of chronic gingivitis and chronic periodontitis.
3. Smoking has a suppressive effect on PTX3 level in GCF of smokers.

Table 1: Comparison of clinical parameters of smoker and non-smoker sub-groups.

		Clinical parameters		
		GI Range M ± SD	PPD Range M ± SD	CAL Range M ± SD
Smoker gingivitis sub-group (SG)		1 – 2 1.6 ± 0.516	2 – 2.86 2.396 ± 0.327	-
Non-smoker gingivitis sub-group (NSG)		2 – 3 2.3 ± 0.483	1.3 – 2.4 1.804 ± 0.413	-
Smoker periodontitis sub-group (SP)		1 – 2 1.4 ± 0.516	4.1 – 7.7 5.735 ± 1.258	3.5 – 6.6 4.93 ± 1.020
Non-smokes periodontitis sub-group (NSP)		2 – 3 2.4 ± 0.516	3.1 – 5.9 4.580 ± 0.597	2.75 – 4.6 3.62 ± 0.871
T-test	(SG) vs (NSG)	3.13	-	-
	(SP) vs (NSP)	4.33	2.44	3.23
P value	(SG) vs (NSG)	0.006**	-	-
	(SP) vs (NSP)	0.000***	0.025*	0.005**

T- test, \* P ≤ 0.05, \*\* P ≤ 0.01, \*\*\* P ≤ 0.001, PPD: probing pocket depth, GI: gingival index, CAL: clinical attachment loss.

Table 2: Comparison between PTX3 levels in both smoker and non-smoker groups and sub-groups.

		Rage	Mean±SD	P value
Gingivitis sub-groups	SG	0.3-0.79	0.444±0.144	0.009**
	NSG	0.4-0.84	0.656±0.031	
Periodontitis sub-group	SP	0.46-1.84	0.965±0.475	0.006**
	NSP	0.9-2.8	1.74±0.615	
Study group	S	0.07-1.84	0.517±0.444	0.017*
	NS	0.2-2.8	0.896±0.721	
Control group	+veC	0.07-0.22	0.142±0.051	0.000***
	-veC	0.2-0.45	0.292±0.068	

T- test, \* P ≤ 0.05, \*\* P ≤ 0.01, \*\*\* P ≤ 0.001, SG: smoker gingivitis, SP: smoker periodontitis, NSG: non-smoker gingivitis, NSP: non-smoker periodontitis, +veC: positive control, -veC: negative control, S: smoker group, NS: non-smoker group.

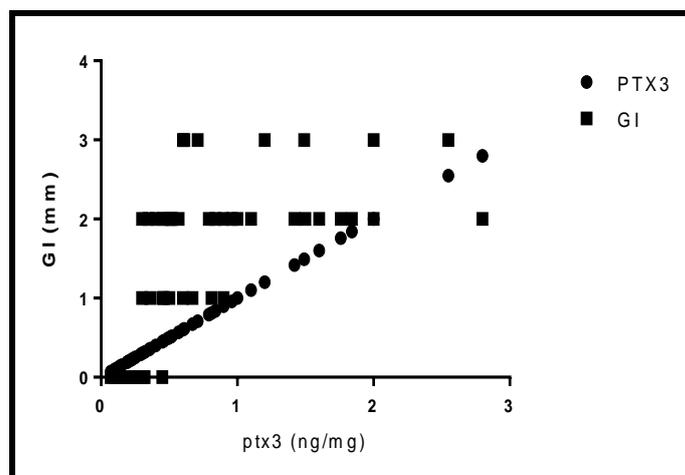


Figure 1: Correlations between PTX3 levels and GI.

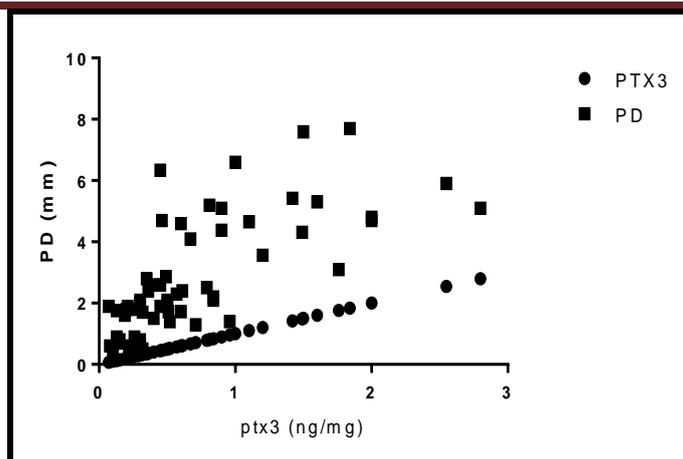


Figure 2: Correlations between PTX3 levels and PPD.

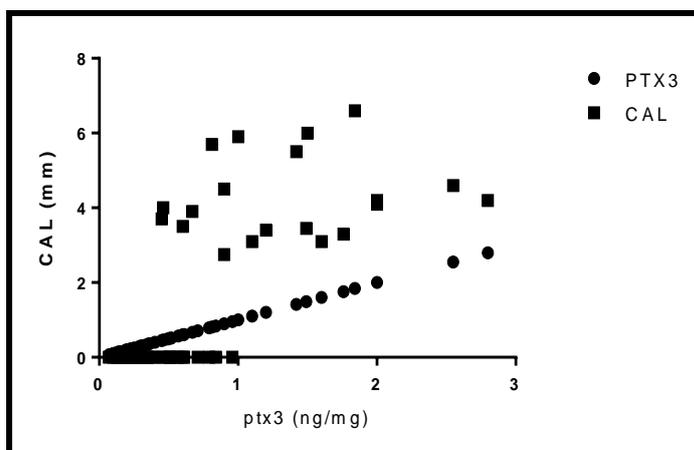


Figure 3: Correlations between PTX3 levels and CAL.

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