Correlation of periodontal ligament associated protein-1 and tumour necrosis factor – alpha levels in gingival crevicular fluid and serum in periodontal health and diseased subjects before and after non-surgical periodontal therapy

Ankitha Menon[1], Athira Jayan[1], AR Pradeep[1]

[1]The Oxford Dental College, Department of Periodontology, Bengaluru, Karnataka.

Abstract

Aim: The present study was undertaken to evaluate the concentration of Periodontal Ligament Associated Protein-1 (PLAP-1) and Tumour Necrosis Factor – Alpha (TNF-α) in Gingival Crevicular Fluid (GCF) and serum in healthy, periodontitis (P) and P participants after the Non-Surgical Periodontal Therapy(NSPT).

Materials and Methods: 26 participants with age (25-50yrs) was included in study. Based on clinical and radiological parameters these participants were divided as; group 1: healthy, group 2: P, group 3: P after treatment. PLAP-1 and TNF-α levels in GCF and serum, periodontal parameters like Plaque Index (PI), Gingival Index (GI), Probing Pocket Depth (PPD),Clinical Attachment Level (CAL) were measured at baseline and 4-6 weeks after NSPT.

Results: The results of the study indicated that the mean PLAP-1 increased in P participants (2842.56±1004.72pg/ml & 2292.63±495.01) and TNF-α (15.80±0.71pg/ml &13.98±0.3pg/ml) levels in both GCF and serum .In treated P group there was statistically significant reduction in PLAP-1 (927.38±266.59pg/ml and 975.94±221.88pg/ml) and TNF-α (3.73±0.20pg/ml and 4.09±0.59pg/ml) concentration in both GCF and serum respectively.

Conclusion: There is substantial increase in the concentration of PLAP-1 & TNF-α in GCF and serum in periodontitis. Treatment of periodontal disease leads to a reduction in levels of PLAP-1 & TNF-α.

Keywords: Periodontal Ligament Associated Protein-1; Tumour Necrosis Factor α; periodontitis; non-surgical periodontal therapy; Gingival crevicular fluid.

Introduction

Periodontitis is a complex infectious disease with several etiologic and contributory factors. The site-specificity of periodontal breakdown can't be explained by mere plaque accumulation, and not even by bacterial specificity or immunopathology taken in isolation, but possibly by a combined effect of all (Haffajee and Socransky, 1994). Proinflammatory cytokines activate M1 ('killer') macrophages within the immune defense against infectious agents, but prolonged release or overproduction of proinflammatory cytokines may additionally activate osteoclasts and matrix metalloproteinases (collagenases) (Page and Schroeder, 1976; Hajishengallis, 2015). The expression of gene profile describing quantitative aspects of the genes active within the human PDL identified a new gene, PLAP-1

Correspondence to: Ankitha Menon
E-mail: todcperiodontics@gmail.com

(periodontal-ligament-associated protein-1), which is predominantly expressed within the periodontal tissue. It was named *Asporin*, because of its unique aspartic stretch at the N terminus of the translated open reading frame (Yamada *et al*., 2006).

PLAP-1/asporin inhibited bone morphogenetic protein-2 induced cell differentiation probably by binding to Bone morphogenic protein-2 (BMP-2) directly. (Tomoeda M *et al*., 2008) Asporin inhibits Tumour Growth Factor-beta (TGF-b) mediated expression of cartilage matrix genes and chondrogenesis. TGF-b is a multifunctional cytokine involved in numerous essential biological processes, including development, Extra cellular matrix (ECM) synthesis, cell proliferation and differentiation, and tissue repair (Kou, Nakajima and Ikegawa, 2010). Degradation of periodontal ligament collagen fibers might favor inflammatory mediator infiltration within the periodontium. Reduced PLAP-1 expression and destruction of periodontal ligament collagen within the periodontal ligament might increase the chance of periodontal destruction (Yamada *et al*., 2001).

Tumor necrosis factor (TNF) is a critical cytokine, which contributes to both physiological and pathological processes. TNF-α is principally produced by macrophages. TNF-α is a key mediator of both acute and chronic systemic inflammatory reactions. TNF not only induces its own secretion, but it also stimulates the production of other inflammatory cytokines and chemokines (Farajzadeh, Karimi-Gharigh and Dastmalchi, 2017). TNF-α is a proinflammatory cytokine known for its substantial role in periodontitis mediated bone loss and is detected in saliva and gingival crevicular fluid (GCF) both in health and periodontitis. Increased concentration observed in periodontitis correlate closely with the tissue destruction and immunologic response. These can, in turn, induce an elevated expression of matrix metalloproteinases (MMP) in periodontal tissues. This impairs the conventional host response in bacterial clearance and neutralizing of infection. The granulocyte function is impaired, these cells react to a bacterial challenge by releasing the serine proteases elastase and matrix metalloproteinase to which are associated with degradation of connective tissue (Singh, Gupta, Bey and Khan, 2014).

Thus this study aimed to evaluate the levels of PLAP-1 and TNF-α in GCF and serum in healthy and periodontally affected individuals and after NSPT.

Materials and Methods

Ethical consideration

The ethical clearance was obtained from the Institutional Committee and Review Board, The Oxford Dental College, Bangalore (Ref: 240/2018-19). The study was conducted in accordance with Helsinki Declaration, 1975. Informed consent was obtained from participants of study.

Participants of study

The study population consisted of 26 participants (10 healthy, 16 periodontitis before and after treatment) attending the outpatient section, Department of Periodontology, The Oxford Dental College, Bangalore. Periodontitis was diagnosed based on the criteria of 2017 world workshop classification of periodontal disease (Caton *et al*., 2018). Inclusion criteria were: 1) Age group 25 – 50 years; 2) Participants with diagnosis of periodontitis with staging including II and III and grade A and B according to world workshop Classification of Periodontal Diseases 2017 (Caton *et al*., 2018); 3) Participants should have at least 20 natural teeth. Exclusion criteria are as follows: 1) Smokers and alcoholics; 2) Pregnant and lactating females; 3) Participants with Diabetes mellitus, hypertension, heart diseases, rheumatoid arthritis, tumors; 4) Participants on any medication like phenytoin, cyclosporine, and calcium channel blockers; 5) Participants who have received anti-inflammatory drugs, antibiotics and any periodontal treatment in the previous six months; 6) Any other systemic disease which can alter the course of periodontal disease; 7) Post-menopausal women.

Study design

A total of 26 participants were recruited in the study. Sample size was determined based on the power analysis at confidence interval of 91% ($p \leq 0.05$)

The study included following three groups:

» Group 1 (Healthy): Consisted of 20 samples from 10 Participants (10 GCF and 10 Serum) with clinically healthy periodontium with no evidence of disease and gingival index≤1, probing pocket depth ≤3mm and clinical attachment level≤0.

» Group 2 (P): Consisted of 32 samples from 16 Participants (16 GCF and 16 Serum) who showed clinical signs of gingival inflammation and gingival index-≥1,bleeding on probing, probing pocket depth ≥5mm and clinical attachment level ≥3mm, with radiographic evidence of bone loss. Stage II & III and Grade A & B.

» Group 3 (P after NSPT): Consisted of 32 samples (16 GCF and 16 Serum) from group 2 Participants diagnosed as periodontitis who received non-surgical periodontal therapy (NSPT).

Periodontal examination

Participants were selected for each group after detailed and precise case history recording and radiographic examination. The clinical parameters were recorded at baseline (at first visit) and 4 to 6 weeks after treatment.

UNC-15 periodontal probe was used to measure periodontal probing depth (PPD) and clinical attachment level (CAL), Plaque index (PI) (Silness and Loe, 1964), Gingival index (GI) (Loe and Silness, 1963) were measured in all Participants. Bleeding on probing was checked in all participants to understand the severity of disease but they weren't considered for analysis.

GCF collection

The GCF sample were collected the following day after the clinical parameters were recorded to avoid contamination with blood. GCF was collected from the site with highest probing depth and bone loss. Following NSPT after 4–6 weeks, GCF samples were collected from the same site as baseline. The selected site was air dried and isolated with cotton rolls. Supragingival plaque was removed without touching the marginal gingiva to avoid contamination and blocking of the microcapillary pipette. There was no other restrictions advised to patient prior to GCF collection. GCF was collected by placing the 5µl microcapillary pipette by Sigmund Aldrich Company USA extracrevicularly, until it gently touched the marginal gingiva, a standardized volume of 3µl was collected in 10-20 minutes from test site. GCF samples were stored in a freezer, temperature maintained at -70^{0}C till the assay procedure was carried out (Björn, Koch and Lindhe 1965; Sueda, Bang and Cimasoni, 1969).

Serum collection

The serum sample were collected the following day after the clinical parameters were recorded along with GCF. 2ml of blood was collected from the antecubital fossa by venipuncture using 20-gauge needle with 2ml syringe and was transferred to a vial. Before centrifugation, for 30 minutes vial should be kept in upright position. Centrifugation was done for 15 minutes at 2200-2500 RPM. Serum was transferred to plastic screw cap vial and stored at -70◦C till the assay procedure (Yu *et al*., 2011).

Non surgical periodontal therapy

The Participants included in the study received initial periodontal therapy consisting of patient education and motivation, thorough oral hygiene instructions and full mouth supragingival and subgingival scaling and root planing with Gracey curettes and ultrasonic scaler under topical anesthesia as required. Participants were put on oral hygiene maintenance which included oral hygiene instructions and recall after 4 - 6 weeks and GCF and serum was collected. NSPT was provided by same dentist who did the examination of participants.

PLAP-1 concentration was determined from these samples using Enzyme Linked Immuno Sorbent Assay (ELISA) kit that is Human ASPORIN ELISA kit obtained from Kreative Technolabs, USA (Catalogue no. KTL11403). TNF-α concentration was determined from these samples using ELISA kit that is Human TNF-α GENLISA™ ELISA kit obtained from Krishgen Biosystems, INDIA (Catalogue no. KB1145).

Statistical analysis

All data obtained are presented as the mean ± standard deviation (SD). Statistical comparison between groups was performed using Kruskal-Wallis test and one way analysis of variance (ANOVA) was performed for statistical analysis. P-value <0.001 were considered statistically significant.

Results

Clinical findings

Table 1 indicates age and gender distribution among study groups. There was no significant difference in gender, among the three groups. But when comparing age among groups it showed statistically significant difference. As expected, all the periodontal parameters were significantly higher in periodontitis participants than those in healthy controls. The periodontal parameters were reduced after NSPT and it was statistically significant (Table 2).

Table 1. Age & Gender distribution among study groups.

Variable	Category	Group 1		Group 2		*p*-value
		Mean	*SD*	*Mean*	*SD*	
Age	Mean and SD	31.60	4.27	42.94	5.98	<0.001*[a]
	Range	26 - 38		30 – 50		
		n	***%***	n	*%*	
Sex	Males	4	40.0%	11	68.8%	0.15[b]
	Females	6	60.0%	5	31.3%	

[a]: Statistical analysis for age distribution was done by Mann-Whitney test. [b] : Statistical analysis for gender distribution was done by chi square test. p <0.05. n : number of Participants. Group 1 - Healthy Patients, Group 2 - Periodontitis Patients.

Table 2. Comparison of mean values of different clinical parameters between the three groups.

Variables	Groups	n	Mean	SD	Min	Max	*p*-value[a]
PI	Group 1	10	0.45	0.10	0.3	0.6	<0.001*
	Group 2	16	1.67	0.22	1.2	2.1	
	Group 3	16	0.73	0.18	0.4	1.0	
GI	Group 1	10	0.41	0.10	0.3	0.5	<0.001*
	Group 2	16	1.76	0.26	1.3	2.3	
	Group 3	16	0.83	0.16	0.6	1.1	
PPD	Group 1	10	2.66	0.47	2.0	3.6	<0.001*
	Group 2	16	5.74	0.44	5.0	6.7	
	Group 3	16	4.48	0.58	3.8	5.9	
CAL	Group 1	10	0.00	0.00	0.0	0.0	<0.001*
	Group 2	16	3.53	0.39	3.1	4.1	
	Group 3	16	2.63	0.37	1.9	3.1	

* Statistically Significant. [a] comparison of mean PI. GI. PPD, CAL was performed using Kruska-Wallis test. p-value <0.05. PI- Plaque Index, GI – Gingival Index, PPD – Probing Pocket Depth, CAL – Clinical Attachment Level, SD- Standard Deviation.

Levels of PLAP-1 in serum and GCF among healthy participants, P participants and post NSPT participants

Table 3 and Figure 1 demonstrates mean GCF PLAP-1 levels for all the groups. On comparing, group 2 demonstrated significantly higher GCF PLAP-1 levels 2842.56 ± 1004.72 than in group 1 (1451.30 ± 123.35) at p<0.001. The mean PLAP-1 values for the post treatment group 3 were reduced to 927.38 ± 266.76 as compared to group 2 and group 1 at p-value <0.001. PLAP-1 concentration in serum which was maximum in group 2 that is 2292.56 ± 495.01 and minimum in post treatment group was 3 975.94 ± 221.88 statistically significant differences was found in levels of PLAP-1 in both GCF and serum before and after NSPT.

Levels of TNF-α in serum and GCF among healthy Participants, P Participants and post NSPT Participants

In Table 4 and Figure 2 Group 2 showed significantly higher GCF TNF-α levels 15.80 ± 0.71 group 1 (6.31 ± 0.74) at p<0.001. The mean TNF-α values for the post treatment group 3 were reduced to 3.73 ± 0.20 as compared to group 2 and group 1 at *p*-value <0.001. However TNF-α concentration in serum demonstrated maximum in group 2 that is 13.98 ± 0.30 and minimum in post treatment group was 3that is 4.09 ± 0.59.

Tables 5 and 6 demonstrates stepwise multiple linear regression analysis for GCF & serum PLAP-1 and TNF-α levels is done in overall samples. Table 5 demonstrates multiple linear regression analysis of GCF PLAP-1 levels and coefficient value for GI is 2033.69 and CAL is 858.65 which is statistically significant at p= <0.001 and also the coefficient value for PPD is 587.04 which is statistically significant at p=0.01. This indicates that for every 1mm increase in CAL, PPD and every 1 score increase in gingival index there will be 858.65, 587.04, and 2033.69 increase in GCF PLAP-1 levels respectively. The R^2 which is the predicting potential of particular model and for overall it is for GCF PLAP-1 levels is 0.76. Thus, GI, CAL, PPD are the predictors in overall samples. The coefficient value for GI is 1394.36 and CAL is 564.10 for serum PLAP-1 which is statistically significant at p= <0.001 and also the coefficient value for PPD is 319.78 which is statistically significant at p=0.03. This indicates that for every 1mm increase in CAL, PPD and every 1 score increase in gingival index there will be 564.10, 319.78, and 1394.36 increase in serum PLAP-1 levels. The R^2 which is the predicting potential of particular model and for overall it is for serum PLAP-1 levels is 0.76. Thus, GI, CAL, PPD are the predictors in overall samples.

Table 6 indicates multiple linear regression analysis for GCF TNF-α levels the test results demonstrated that coefficient value for PI is 6.09, GI is 7.12 and CAL is 2.26 which is statistically significant. This indicates that for every 1mm increase in CAL and every 1 score increase in GI, PI there will be 2.26, 7.12, and 6.09 increase in GCF TNF-α levels. The R^2 which is the predicting potential of particular model and for overall it is for GCF TNF-α levels is 0.87. Thus, GI, CAL, PI are the predictors in overall samples. In multiple linear regression analysis for serum TNF-α levels the test results demonstrated that coefficient value for PI is 4.32, GI is 5.96, CAL is 3.68 and PPD is 1.78 which is statistically significant. This indicates that for every 1mm increase in CAL, PPD and every 1 score increase in GI, PI there will be 3.68, 1.78, 4.32 and 5.96 increase in serum TNF-α levels. R^2 which is the predicting potential of particular model and for overall it is for serum TNF-α levels is 0.85. Thus, GI, CAL, PI, PPD are the predictors in overall samples.

Table 3. Comparison of mean values of PLAP 1 levels [in pg/ml] between the three groups.

Variables	Groups	n	Mean	SD	Min	Max	*p*-value[a]	Sig. Diff	*p*-value[b]
PLAP 1_ GCF	Group 1	10	1451.30	123.35	1314	1690		G1 Vs G2	<0.001*
	Group 2	16	2842.56	1004.72	1834	5343	<0.001*	G1 Vs G3	<0.001*
	Group 3	16	927.38	266.76	325	1173		G2 Vs G3	<0.001*
PLAP 1_ SERUM	Group 1	10	1464.00	155.42	1313	1689		G1 Vs G2	<0.001*
	Group 2	16	2292.63	495.01	1822	3332	<0.001*	G1 Vs G3	<0.001*
	Group 3	16	975.94	221.88	534	1192		G2 Vs G3	<0.001*

*Statistically Significant. Comparison of mean PLAP-1 value was done using [a]Kruskal-Wallis test followed by. [b]Mann-Whitney's post-hoc analysis. p-value <0.05. PLAP-1 Periodontal ligament associated protein – 1, GCF – gingival crevicular fluid, SD – Standard Deviation.

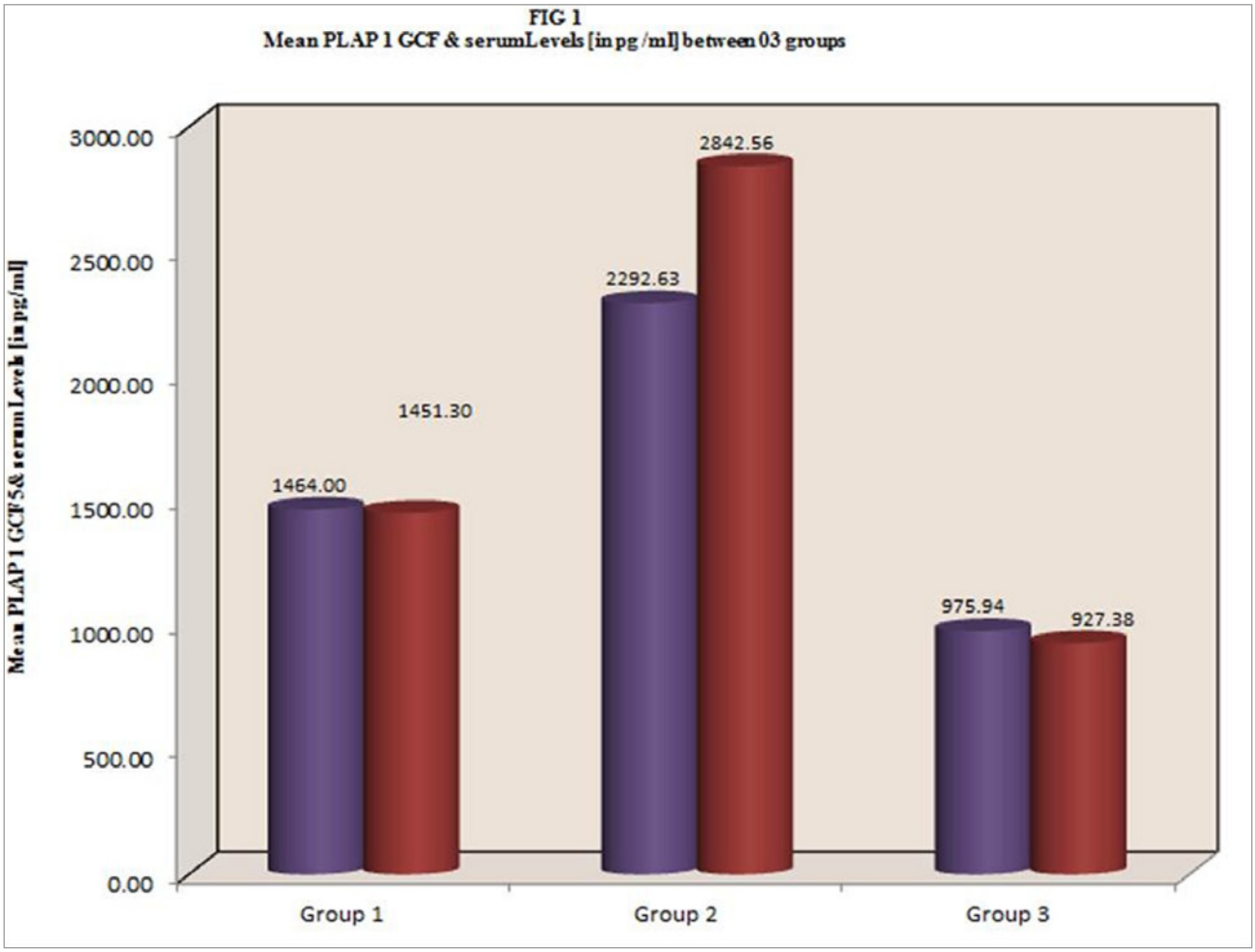

Figure 1. Mean PLAP 1 GCF & serum Levels [in pg /ml] between the three groups: group 2 demonstrated significantly higher GCF PLAP-1 levels than in group 1 at p<0.001. The mean PLAP-1 values for the post treatment group 3 were reduced compared to group 2 and group 1 at p-value <0.001. PLAP-1 concentration in serum which was maximum in group 2 and minimum in post treatment group 3 PLAP-1 GCF levels PLAP-1serum levels.

Table 7 demonstrates normality of the data which was accessed using Shapiro-Wilk test. The null hypothesis for this test is that the data are normally distributed.

Discussion

The present study is designed to evaluate the correlation of PLAP-1 and TNF-α concentration in GCF and serum with clinical parameters in periodontal health and disease and also to assess the effect of non-surgical periodontal therapy on PLAP-1concentration. Yu *et al*. examined the relationship between PLAP-1 and osteoclastogenesis in experimental periodontitis. Acute periodontal inflammatory infiltration and alveolar bone destruction were induced by TNF-α administration

Table 4. Comparison of mean values of TNF-α levels [in pg/ml] between the three groups.

Variables	Groups	n	Mean	SD	Min	Max	*p*-value[a]	Sig. diff	*p*-value[b]
TNF-α GCF	Group 1	10	6.31	0.74	5.5	7.8	<0.001*	G1 Vs G2	<0.001*
	Group 2	16	15.80	0.71	14.1	16.8		G1 Vs G3	<0.001*
	Group 3	16	3.73	0.20	3.5	4.2		G2 Vs G3	<0.001*
TNF-α SERUM	Group 1	10	7.80	0.59	6.2	8.2	<0.001*	G1 Vs G2	<0.001*
	Group 2	16	13.98	0.30	13.4	14.6		G1 Vs G3	<0.001*
	Group 3	16	4.09	0.59	3.5	5.8		G2 Vs G3	<0.001*

* - Statistically significant. Comparison of mean TNF-α value was done using [a]One way ANOVA test followed by [b]Tukey's post-hoc analysis. p-value <0.05. TNF-α – Tumour necrosis factor - alpha, GCF – gingival crevicular fluid, SD – Standard deviation.

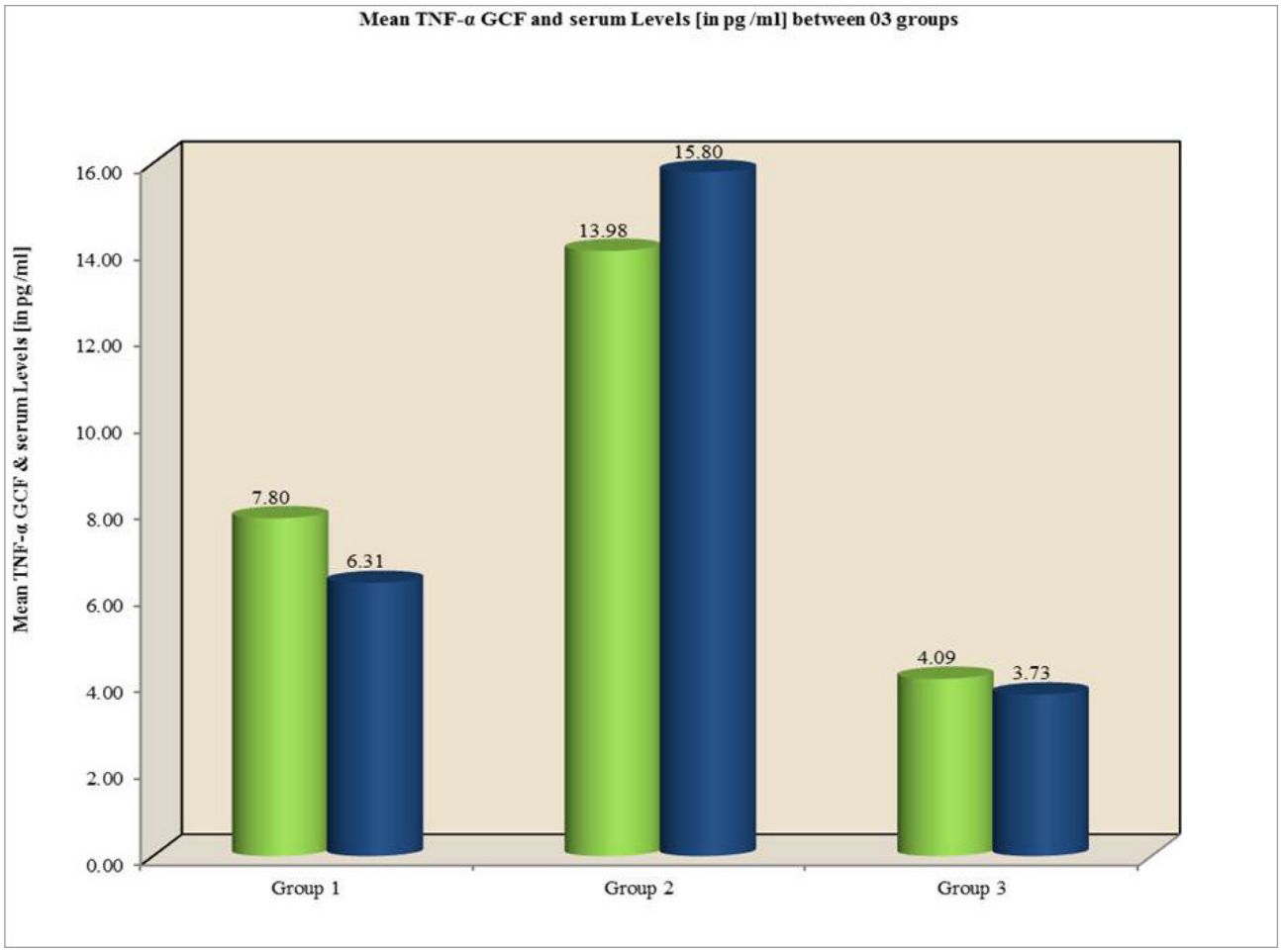

Figure 2. Mean TNF-α GCF and serum Levels [in pg /ml] between the three groups: Group 2 showed significantly higher GCF TNF-α levels than group 1 at p<0.001. The mean TNF-α values for the post treatment group 3 were reduced compared to group 2 and group 1 at p-value <0.001. TNF-α concentration in serum demonstrated maximum in group 2 and minimum in post treatment group 3
TNF-α serum levels ***TNF-α GCF levels.***

in Wistar rats. He concluded that PLAP-1 positive inflammatory cells are involved in the pathogenesis of periodontitis. An increase in PLAP-1 positive inflammatory cell number contributes to periodontal inflammation and alveolar bone loss (Yu X *et al*., 2019).

Yamaba in 2015 conducted a study to assess the regulatory function of PLAP-1 against BMP-2 and, in turn, may influence susceptibility to periodontal disease. Western blotting analysis and luciferase assay confirmed that D14- PLAP-1 suppressed BMP-2 signal transduction. Analysis of these data suggests that the D repeat polymorphism of PLAP-1/asporin has a significant influence on the functions of PDL cells (Yamaba, Yamada and Kajikawa, 2015).

Table 5. Multiple linear regression analysis of PLAP-1 in Over-all samples.

Group	DV	IV	b	SE	t	P-value	R^2
Overall	PLAP 1 GCF	Constant	-1067.83	557.13	-1.917	0.06	0.76
		GI	2033.69	285.19	7.131	<0.001*	
		CAL	858.65	182.59	-4.703	<0.001*	
		PPD	587.04	224.11	2.619	0.01*	
			-43.11	352.64	-0.122		
Overall	PLAP 1 SERUM	Constant	1394.36	180.52	7.724	0.90	0.76
		GI	564.10	115.57	-4.881	<0.001*	
		CAL	319.78	141.86	2.254	<0.001*	
		PPD				0.03*	

* - Statistically Significant. DV – dependent variable. IV – Independent variable. β – beta is the estimated coefficients of explanatory variables. SE – standard error. t – size of difference relative to variation in sample data. R^2 – proportion of variance for dependent variable that's explained by an independent variable. PLAP-1 Periodontal ligament associated protein – 1, GCF – gingival crevicular fluid.

Table 6. Multiple linear regression analysis of TNF-α in over-all samples.

Group	DV	IV	B	SE	T	*p*-value	R^2
Overall	TNF-α GCF	Constant	0.32	0.67	0.476	0.637	0.87
		PI	6.09	1.96	3.099	0.004*	
		CAL	2.26	0.42	-5.437	<0.001*	
		GI	7.12	2.13	3.349	0.002*	
Overall	TNF-α Serum	Constant	-1.57	1.79	-0.875	0.39	0.85
		PI	4.32	1.65	2.622	0.01*	
		CAL	3.68	0.59	-6.195	<0.001*	
		GI	5.96	1.79	3.324	0.002*	

* - Statistically significant. DV – dependent variable. IV – Independent variable. β – beta is the estimated coefficients of explanatory variables. SE – standard error. t – size of difference relative to variation in sample data. R^2 – proportion of variance for dependent variable that's explained by an independent variable. TNF-α – Tumour necrosis factor - alpha, GCF – gingival crevicular fluid.

Table 7. Tests of normality.

		Group	Shapiro-Wilk Test		
			Statistic	n	*p*-value
PLAP 1	GCF (pg/ml)	Group 1	0.916	10	0.325
		Group 2	0.875	16	0.033*
		Group 3	0.821	16	0.005*
	Serum (pg/ml)	Group 1	0.824	10	0.028*
		Group 2	0.833	16	0.008*
		Group 3	0.857	16	0.018*
TNF-α	GCF (pg/ml)	Group 1	0.833	10	0.036*
		Group 2	0.896	16	0.070
		Group 3	0.875	16	0.033*
	Serum (pg/ml)	Group 1	0.589	10	<0.001*
		Group 2	0.945	16	0.420
		Group 3	0.834	16	0.008*

* - Statistically Significant. Normality of the data which was accessed using Shapiro Wilk test. p-value < 0.05.

According to Xu in the year 2015 increased levels of PLAP-1 was seen in osteoarthritis, fatty liver conditions, and cancer (Xu L, 2015).

Gokul K in 2012 demonstrated an increased concentration of TNF-α in GCF and serum of chronic periodontitis as compared to those of healthy individuals but the effect of periodontal treatment on TNF-α concentration was not evaluated (Gokul, 2012). Turer in 2016 had also evaluated the interplay of the angiogenic factor Vascular Endothelial Growth Factor (VEGF) and the pro-inflammatory mediator TNF-α with respect to the action of endocan in chronic periodontitis (P) patients before and after NSPT. Thus, in our study inclusion of post treatment group has

helped us to evaluate the effect of periodontal therapy on TNF-α concentration (GCF 3.73±0.20pg/ml and SERUM 4.09±0.59pg/ml) which can further confirm its role in periodontal disease. This was in accordance with the study conducted by Turer. The ELISA kit used in this study allowed accurate quantitative evaluation of PLAP 1 and TNF-α with high sensitivity and specificity, which was also in accordance with study by Turer (Türer, Durmuş, Balli and Güven, 2017). On correlating PLAP 1 with TNF-α, Yu *et al*. in 2018 conducted a study to clarify the roles of PLAP-1 in osteoclastogenesis in rat experimental periodontitis; acute periodontal inflammatory infiltration and alveolar bone destruction were induced by TNF-α administration. Thus, TNF-α increased the number of PLAP-1- positive inflammatory cells, which contributed to the periodontal inflammation and alveolar bone loss (Yu X *et al*., 2019).

TNF-α is related with the inflammatory condition of the periodontium which is also explained by increased expression of inflammatory cells such as fibroblasts, endothelial cells or infiltrating leukocytes i.e. mononuclear cells, macrophages and neutrophils which are responsible for secretion and synthesis of TNF-α (Stashenko, Jandinski, Fujiyoshi, Rynar and Socransky, 1991).

Here the group 1 showed increased PLAP-1 and TNF-α levels compared to group 3; even though the exact reason is unknown it might be due to underlying subclinical inflammation.

GCF volume increases with inflammation, and declines following resolution of inflammation with NSPT. The production of cytokines such as TNF-α appears to play a central role in the progressive migration of an inflammatory front toward the alveolar bone. This suggests that the production of cytokines at deeper levels within the gingival connective tissue leads to an inflammatory cascade in this area. Once a "critical level" of proinflammatory cytokine TNF-α production is reached, a physiologic response becomes a pathologic response. If the inflammatory front occurs predominantly in the area of attachment to cementum, the result will be loss of attachment. If it occurs near the alveolar crest, the result would be a loss of bone. Offenbacher has stated that serum also provides information about periodontal pathogen-induced inflammation and responses. A literature review showed that biomarkers in serum may be linked to PD. In GCF, inflammation-related molecules may provide reliable information about the status of PD. Increased levels of TNF-α in serum was due to elevated levels of TNF-α in GCF i.e., systemic "Spill" of cytokine via the circulation as reported by Offenbacher (Offenbacher, 1996).

In this study a positive correlation was found between GCF PLAP-1 levels with serum PLAP-1 levels and serum and GCF TNF-α levels in all groups when it was assessed with Spearman correlation test. The concentration of PLAP 1 and TNF-α in the GCF and serum increases with the increasing severity of periodontal disease. However in this study results were not calculated according to stages and grades of patients. Further NSPT was done to arrest the progression of periodontal disease and this resulted in significant reductions of PLAP 1 & TNF-α concentration in GCF and serum. However further, longitudinal, prospective, multicentred, interventional studies involving larger population should be carried out to validate the findings of the present study and to better understand the role of PLAP 1 and TNF-α in the pathogenesis of periodontal diseases.

Conclusion

PLAP-1 & TNF-α is present in GCF and serum in periodontal health and disease. There is substantial increase in the concentration of PLAP-1 & TNF-α in GCF and serum in periodontitis. Treatment of periodontal disease leads to a proportional reduction in GCF and serum levels of PLAP-1 & TNF-α.

Conflict of Interest and Sources of Funding

The authors declare no conflicts of interest and no external funding.

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