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Research Article

ASSOCIATION OF DOSE DEPENDENT EFFECTS OF SMOKING AND CHRONIC PERIODONTITIS BY ESTIMATING ELASTASE LEVELS

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A B S T R A C T

Background: To determine the association of dose dependent effects of smoking and chronic periodontitis by estimating elastase levels in Saliva, GCF and serum samples.
Materials and Methods: 125 male subjects in the age group of 25-55 years were included and grouped as, Group A, Periodontally healthy non-smokers Group B, Periodontally healthy smokers. Group C, Non smokers with chronic periodontitis. Group D, Chronic periodontitis smokers (< 10 Cigarettes/day) and Group E, Chronic periodontitis smokers (> 10 Cigarettes/day). Smoking history (pack years), Clinical parameters PI, GBI, PPD CAL, Salivary, GCF and Serum Elastase levels were recorded.
Results: Clinical parameters and Elastase activity in Saliva, GCF and Serum showed significant difference between diseased group and healthy group. In between the diseased group comparison, GCF and Saliva showed Lower Elastase activity in smoking periodontitis group.Whereas, Serum showed higher Elastase activity. Also, it was noted that, GCF and serum elastase activity were higher in patients with greater than 10 pack years.
Conclusion: Elastase proves to be a reliable marker not only in diagnosing the periodontal disease severity locally but also systemically. It further helps in prediction and management of the disease.

INTRODUCTION

Periodontitis is a common inflammatory disease associated with gram-negative anaerobic bacteria present in the dental biofilm which leads to irrevocable impairment of periodontium[1]. The interaction of the host immune system with plaque bacteria has been implicated in the pathophysiology of chronic periodontal disease. The local host response to these bacteria involves the recruitment of leukocytes and subsequent release of inflammatory mediators and cytokines that plays a critical role in destroying host tissues. The interplay between chemokines, pathogenic microorganisms and the host inflammatory cells leads to inflammation, irreversible attachment loss, bone destruction, and eventually tooth loss. Statistics present the grim reality that 95% of the population in India suffer from periodontal disease[2].

Infection is a mandatory pre-requisite for periodontitis, although, there exist a multifactorial risk pattern including bacterial challenge, smoking, age, gender, diabetes, and socioeconomic and genetic factors. Cigarette smoking is a

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Smoking affects the immune system by impairing host response by inhibiting granulocyte function and by neutrophil respiratory burst which causes oxidative stress in tissues. Smoking has two-sided effect on periodontal inflammation. One is it has an effect on oxygen depletion with tissue damage and other is it impairs the ability of neutrophils to response to subgingival periodontal bacteria. Nicotine in smoke seems to play an important role in host immune modulation. Reduced chemotaxis and impaired phagocytosis in smokers have suggested that smokers periodontal defence is defective compared with non- smokers[3]. These changes are reflected systemically in serum and locally in saliva and GCF.

Studies prove that, Smoking is associated with a two to eight fold increased risk for periodontal attachment and bone loss, depending on the definition of disease severity and smoking dose[4,5]. Cross-sectional studies have shown that smokers are two to seven times more likely to present periodontitis, compared to nonsmokers[8,9]. In longitudinal studies, smokers developed more sites with increased PD and alveolar bone loss[10]. In addition, responses to periodontal treatment, both non-surgical and surgical, appear to be compromised[11-13]. Treatments in smokers resulted in lesser probing depth reduction and smaller clinical attachment level (CAL) gain.

Moreover, smokers were at a higher risk for recurrent diseases during periodontal maintenance care than non-smokers[14].

The effect of cigarette smoking on the severity of the periodontal disease depends on the dose dependent relationship. Smoking is then quantified as a composite value (pack year) of the number of packs of cigarettes smoked per day multiplied by number of years smoked. It was showed that the proportion of current smokers was increased as the severity of periodontitis increased and showed a positive correlation between the level of cigarette consumption and the severity of periodontitis. The more the cigarettes consumed (in terms of pack year), the worse periodontal condition was observed[6,7].

Tobacco smoking leads to significant increase in the circulating burden of Neutrophil Elastase and MMPs in humans. The enzyme is capable of degrading a large spectrum of various molecules in human tissues, including periodontal tissues, such as Collagen, Laminin, Fibronectin, Proteoglycans and Elastin[15].

It is a neutral serine proteinase stored in the cytoplasmic azurophil granules of neutrophilic granulocytes in amounts ranging upto 3 pico grams per cell. It participates intracellularly in phagocytosis, but it can be released extracellularly by triggered granulocytes, together with free oxygen radicals.

Elastase levels are by far the highest of any proteinase quantitated in gingival crevicular fluid during periodontal inflammation[16]. It has been suggested that elastase may be a potential indicator of periodontal disease and disease progression[17,18].

Ingman *et al* **(1993)19** found higher levels of protease, collagenase and elastase in the Saliva of Aggressive periodontitis patients in comparison to Localised Juvenile Periodontitis patients and the healthy controls. Also, scaling and root planning reduced the levels of elastase in Aggressive periodontitis patients.

The molecular markers of tissue destruction in serum or plasma are the manifestations of periodontal diseases are mainly sought to clarify the possible interactions between periodontitis and various systemic diseases and/conditions. Serum or plasma provides information about the inflammatory stimulus and/or response generated in circulation towards the periodontal pathogens that colonize in the subgingival area. Elastase is a host derived enzyme, which is reflected in the serum and plasma.

Elastase is present in saliva, GCF and serum, which can be used as biomarkers for periodontal disease and can be biochemically assayed from these sources. So the Aim of the present study was to determine the levels of Elastase in Saliva, GCF and serum in smokers and non-smokers with chronic periodontitis along with determining the severity of periodontal disease correlating with the smoking dose (pack years).

MATERIALS AND METHODS

This study was conducted in Division of Periodontia, Rajah Muthiah Dental College and hospital in association with Medical Biochemistry, Annamalai University, Chidambaram. The study was approved by Institutional Human Ethical Committee of Rajah Muthiah Medical College and written informed consent from the patients was obtained prior to the initiation of the study.

The study included 125 male subjects in the age group of 25-55 years and divided into five groups, 25 subjects in each group.

Group A included 25 Periodontally healthy non-smokers.
Group B included 25 Periodontally healthy smokers.
Group C included 25 non smokers with chronic periodontitis.
Group D included 25 Chronic periodontitis smokers (< 10 Cigarettes/day).
Group E included 25 Chronic periodontitis smokers (> 10 Cigarettes/day).

Inclusion Criteria : were Smoking and non-smoking Chronic Periodontitis patients in the Age Group of 20-55 years, subjects with atleast 15 natural teeth with 5- 28 sites with probing pocket depth >5mm, with no systemic disease and no previous history of any periodontal therapy that might influence their periodontal condition.

Exclusion Criteria: were, Female subjects, Subjects who had <22 permanent teeth, Former smokers who had quit smoking, Subjects with systemic disorders or any history of systemic antibiotic therapy or any other drug for or within last 6 months and subjects with habits such as Alcoholism.

Clinical Examination

All the patients were seated in the dental chair and a detailed medical and smoking history was recorded following which a thorough Clinical examination was carried out using mouth mirror, a dental explorer and William's Periodontal probe and the following clinical parameters were recorded in proforma.

1.Plaque index (Silness and Loe 1964)[21]
2. Gingival Bleeding Index (Ainamo and Bay 1975)[22]
3. Probing pocket depth (PPD) (Carranza)[23]
4. Clinical attachment level (Ramjford 1959)[24]

Collection of Samples

Collection of Saliva

The pooled salivary samples were collected on the subsequent day after recording of periodontal clinical parameters and all the subjects were advised to rinse mouth with sterile water to remove all loosely adherent food debris from the tooth surface. Saliva was collected every 60 seconds to yield a total of 5ml of each sample in a sterile BD vacutainer SST (Fig.1) The salivary samples were centrifuged at 3000 rpm for 15 min and supernatant obtained was immediately frozen at 40°C and stored until required for biochemical analysis**.**

Collection of GCF

GCF samples were collected on the following day after clinical examination to avoid contamination of blood from the gingival crevice. Prior to collection of GCF, patients were advised to rinse with sterile water, and the area was dried and isolated with cotton rolls or gauze. GCF samples were harvested by Intra-crevicular method, using three absorbent paper strips approximately 2mm wide and 7mm long were inserted side by side in the buccal crevice and left in position for 30 seconds from sites with extensive involvement (Fig.2). The central drying strip was then removed and immediately replaced by the collecting strip. The collecting strips were left in place for

five minutes and then removed and transferred to a sterile Eppendorf tube containing 2ml of saline. Samples contaminated with blood or saliva was discarded. Pooled GCF samples were then transferred to laboratory for the estimation of elastase levels.

Collection of Blood

4 ml of venous blood was collected by venipuncture in EDTA (1 mg/ml) coated test tube. EDTA was added and mixed for 10-20 minutes. Serum was separated from blood by centrifugation at 2000- 3000 rpm for 10 min. The extracted serum (1ml) was stored at 4°C until the time of biochemical analysis.

ELISA

Samples were analysed by using commercially available Human Elastase ELISA kit using sandwich technique according to the manufacturer's instruction for the estimation of Elastase. This assay employs an antibody specific for Human Neutrophil Elastase coated on well plate. All the samples and reagents used were brought to room temperature ($20\text{-}25^0$C). 50µl of each standard and sample were added into each well. Wells were covered and were incubated for 2 hours and then Washed five times with 200 µl of Wash Buffer manually. Invert the plate each time and decant the contents, hit 4-5 times on absorbent material to completely remove the liquid. 50 µl of prepared Biotinylated Human Elastase Antibody was added to each well and incubated for 1 hour at room temperature.50µl of prepared streptavidin was added to each well and incubated for 30 minutes. 50µl of chromogen substrate Tetramethylbenzidine was added to each well and incubated for 7mins or until the optimal blue color density has developed. 50µl of 0.5 N hydrochloric acid (stop solution) was added to each well and color change was observed from blue to yellow. The intensity of color was measured at 450nm immediately.

RESULTS

Paired sample 't' test was applied to find out the statistical difference between the clinical parameters and Elastase activity in between the groups. For Clinical parameters, comparisons were made in between the groups for Plaque scores, Gingival bleeding scores, Probing pocket depths and Clinical attachment levels (Table.1)

The results of the comparison in the clinical parameters showed significant difference between diseased group and healthy group.

In Plaque Score comparison, all groups showed a statistical significance at P=0.00, except C vs E and D vs E.
In Gingival bleeding score comparison, all groups showed a statistical significance at P=0.01, except C vs D and D vs E.
Probing Pocket depth comparison, showed a statistical significance at P=0.05, except C vs D, C vs E and D vs E.
In Clinical Attachment Level comparison, all groups showed a statistical significance at P=0.05, except C vs D and D vs E.
When Elastase activity was compared between groups, Salivary and GCF Elastase showed statistically significant difference between all the groups except D vs E, In contrast Serum Elastase activity showed significant difference between all the groups. (Table.2)

One way analysis of variance was applied for all the above mentioned parameters, and mean and standard deviation was determined, among the different category of Pack Years of smoking periodontitis. (Table.3)

The results showed that, no statistically significant difference in Clinical parameters (PI,GBI,PPD and CAL) among different pack years category.

While comparing the Elastase activity, salivary elastase showed no statistically significant difference, whereas mean GCF Elastase values were found to be higher for the 'Above 10 pack years' group than others and 'F'-value was found to be 2.987 (p-value = 0.050). So it was inferred that 0.05 level significant difference was found in GCF Elastase levels according to their pack years.

Similarly, mean serum elastase values were found to be higher for Above 10 pack years' group than others and 'F'-value was found to be 6.602 (p-value = 0.003). So it was inferred there was 0.01 level significant difference in Serum Elastase levels among different pack years category.

Correlation Analysis

Between the above mentioned parameters and pack years (Table.4) showed that, GCF Elastase levels had significant correlation with CAL (r = -3.50; p = 0.013) and Serum Elastase (r = 0.536; p = 0.00); Salivary Elastase had significant Correlation with Plaque Index (r = 0.427; p = 0.002) and the Pack Years had significant Correlation with GCF Elastase (r = 0.287; p = 0.043) and Serum Elastase (r = 0.410; p = 0.003).

But other clinical parameters (PI, GBI, PPD, CAL) and Salivary Elastase did not have significant correlation with the Pack Years.

DISCUSSION

It is widely accepted that the host response to sub-gingival bacteria plays a critical role in periodontal pathogenesis[25] and that pathogenic processes are modified by environmental and acquired risk factors such as smoking.Experimental evidence accumulated over the last 4 decades has indicated that cigarette smoking is probably a true risk factor for Periodontitis. This environmental exposure has been associated with 2- to 3-fold increases in the odds of developing clinically detectable Periodontitis. Smokers have both increased prevalence and more severe extent of periodontal disease, as well as higher prevalence of tooth loss and edentulism, compared to non-smokers[26]. For example, smokers demonstrate 2.6–6 times increased prevalence of periodontal diseases compared to non-smokers[27] .

In the earlier decades, a multitude of studies investigated the association of smoking status with a variety of periodontal and oral hygiene parameters. These included plaque indices, gingival indices, probing depths, clinical attachment levels, and radiographic alveolar bone levels.

Several studies displayed higher levels of oral debris in smokers than in non-smokers. In our study , Smokers exhibited higher plaque scores when compared to non-smokers in both health and diseased group due to inefficient tooth brushing and increased salivary flow leading to increased calculus formation, which was similar to the study done by **Maddipati Sreedevi *et al* (2011)28**.Cross-sectional investigations have indicated that smokers may present with lower levels of gingival inflammation to a specific level of plaque than non-smokers[29-32]. Smoking exerts a strong, chronic, and dose-

dependent suppressive effect on gingival bleeding on probing. Bleeding on probing was less evident in smokers than non-smokers, indicating its effect on gingival blood vessels[33]. Due to vasoconstrictive action of nicotine, Smokers showed lower gingival bleeding than non-smokers which was similar to the study done by **Goultschin *et al* (1990)34.**

A large amount of data has been gathered on the association of measures of periodontal destruction and cigarette smoking. Probing depths, clinical attachment loss, and alveolar bone loss have all been shown to be both more prevalent and more severe among smokers as compared with non-smoking controls[30,35]. But in our study, there was an increased PPD and CAL levels in both smoking and non-smoking chronic periodontitis patients with that of both healthy controls. When comparison was made between the smoking and non-smoking chronic periodontitis group, there was no significant difference which shows that there was similar disease activity in group C, D & E.

As periodontal disease is characterised by the destruction of the tooth supporting tissues, numerous biochemical constituents in saliva, GCF and serum have been used as a marker of periodontal destruction of which elastase, a serine protease plays a significant role in connective tissue destruction associated with inflammatory process. Its detection would add a new dimension to the measurement of periodontal inflammation.

Neutrophil elastase is one of the most destructive enzymes with the capability of degrading almost all extracellular matrix components as well as plasma proteins and activating pro-MMPs and inactivating TIMP-1. Elastase is released by activated polymorphonuclear leukocytes which degrade collagen, fibronectin, laminin, proteoglycans, etc. Elastase activity is found to be the highest of any protease found in the gingival fluid of gingivitis and periodontitis patients.

A high concentration of NE is stored in azurophilic granules of PMNs, providing an important step in host defence. When activated, NE can be released rapidly into extracellular space and cause local tissue damage. Compared with other enzymes its activity was relatively high in adults with advanced periodontitis. Prior to treatment, elastase activity was on the average of about 30 times as high as it was after treatment. Furthermore, the activity of the elastase was the only one that correlated significantly with the number of 6 mm or deeper periodontal pockets prior to treatment and after initial therapy. Since elastase is an enzyme connected to the destructive phase of inflammation, its detection would add a new dimension to measurement of the periodontal inflammation. Hence, elastase activity was taken as a marker for assessing periodontitis progression and disease activity in the present study.

In our study, Salivary and GCF elastase activity was lower in smoking periodontitis when compared to non-smoking periodontitis group which is is similar to the study done by **Pauletto *et al* (2000)36, Alavi *et al* (1995)37** Whereas serum elastase activity was higher in smoking periodontitis group which is similar to the study done by **Ozaka *et al* (2011)38**.The lower elastase activity in saliva and GCF of smoking periodontitis group inspite of similar clinical parameters may be due to tobacco which causes vasoconstriction and reduced permeability of blood vessels which inhibits neutrophil migration. Because of this, there was abnormal accumulation of neutrophils and macrophages in the inflamed tissues, which rather migrating via the GCF to the oral cavity, gets accumulated in the periodontal tissues and release their constituents causing increased degradation of connective tissue components. So lower elastase activity in smoking periodontitis group cannot be misleaded as having lower disease activity rather it has more periodontal destruction than non-smokers. So if chronic periodontitis is altered by an environmental or systematic factor like smoking, stress or diabetes, it's always best to have both local and systemic sample sources to find out the bio-markers activity. Whereas, There was a significantly higher Serum Elastase activity in smoking chronic periodontitis group when compatred with non-smoking chronic periodontitis group, which may be due to the fact that, though tobacco causes vasoconstriction, there was an increase of about 25% in the number of leukocytes in peripheral blood which causes an abnormal accumulation of neutrophils and macrophages in the inflamed tissues, which was documented in lung tissues by **Mathews J in 200739.**

Earlier studies evidenced a strong association between smoking and advanced periodontal disease. This was also consistent with the hypothesis that smoking has cumulative detrimental effects on periodontal health **(Horning et a1 1992[40])**. In our study we have categorised pack years into three groups as upto 5 years, 5-10 years, and above 10 years.

When the dose response relationship of smoking was compared with that of periodontal clinical parameters, it shows that there was an increase in clinical periodontal parameters when compared with dose response but it was not statistically significant which is similar to the study done by **Gonzalez *et al*[41]**. In their study, they found no statistical significant association between CAL, Bone crest height and the number of cigarettes smoked/day. The reason maybe self-reports given by smokers may not be accurate, the nicotine content of cigarette varies drastically from brand to brand and smoking patterns may vary among different individuals. In addition, individual metabolism, rates of absorption, time of smoking and smoking habits as well as ethnic differences, all play a role in the estimation of tobacco exposure.

Yet another study by **Markkaner *et al* 42** showed a minor association between periodontal disease and number of cigarettes per day and there was a weak positive association between smoking and periodontal parameters. This association could be related to the general poorer state of oral hygiene in smokers, which may in turn altering the vascular tissue and haemodynamics, Whereas most of the studies done by **Guillermo *et al*43, Okamoto44**, there was a strong correlation exists between the smoking and the severity of periodontal destruction.In our study, Pearson correlation test was applied to find out the association between the pack years and salivary, GCF, and serum elastase activity.GCF and serum elastase activity shows the statistical difference between pack years whereas the salivary elastase activity does not show statistical significant difference.

In serum, the increase in elastase activity maybe the possibly because of the fact that, PMN's in smokers could have released elastase prior before reaching the periodontal tissues.

Table 1 Descriptive statistics of periodontal clinical parameters (PI,GBI,PPD,CAL) -groupwise

	Plaque Index		Gingival Bleeding Index		Probing Pocket Depth		Clinical Attachment Level	
Groups	**Mean**	**P value**	**Mean**	**P value**	**mean**	**P value**	**Mean**	**P value**
A vs B	.328 vs.811	0.000	8.317 vs 6.766	0.000	2.121 vs 2.577	0.004	.000 vs.000	-
A vs C	.328 vs1.860	0.000	8.317 vs 74.020	0.000	2.121 vs 5.582	0.000	.000 vs5.946	0.000
A vs D	.328 vs2.235	0.000	8.317 vs 71.629	0.000	2.121 vs 5.860	0.000	.000 vs5.548	0.000
A vs E	.328 vs2.097	0.000	8.317 vs 70.064	0.000	2.121 vs 5.728	0.000	.000 vs5.273	0.000
B vs C	.811vs1.860	0.000	6.766 vs 74.020	0.000	2.577 vs 5.582	0.000	.000 vs5.946	0.000
B vs D	.811 vs2.235	0.000	6.766 vs 71.629	0.000	2.577 vs 5.860	0.000	.000 vs5.548	0.000
B vs E	.811 vs2.097	0.000	6.766 vs 70.064	0.000	2.577 vs 5.728	0.000	.000 vs5.273	0.000
C vs D	1.860 vs2.235	0.011	74.020 vs 71.629	0.142	5.582 vs 5.860	0.217	5.946 vs5.548	0.163
C vs E	1.860 vs2.097	0.140	74.020 vs 70.064	0.027	5.582 vs 5.728	0.539	5.946 vs5.273	0.029
D vs E	2.235vs2.097	0.194	71.629 vs 70.064	0.204	5.860 vs 5.728	0.646	5.548 vs5.273	0.109

Table 2 Descriptive statistics of Salivary, GCF and Serum Elastase -groupwise

	Salivary elastase		Gcf elastase		Serum elastase	
GROUPS	**Mean**	**P value**	**Mean**	**P value**	**mean**	**P value**
A vs B	26.62 vs32.88	0.000	26.62 vs 32.88	0.000	119.79 vs170.72	0.001
A vs C	26.62 vs 102.82	0.000	26.62 vs 102.82	0.000	119.79 vs441.59	0.000
A vs D	26.62 vs50.43	0.000	26.62 vs50.43	0.000	119.79 vs 810.36	0.000
A vs E	26.62 vs 51.71	0.000	26.62 vs 51.71	0.000	119.79 vs 922.08	0.000
B vs C	32.88 vs102.82	0.000	32.88 vs102.82	0.000	170.72 vs441.59	0.000
B vs D	32.88 vs 50.43	0.000	32.88 vs 50.43	0.000	170.72 vs810.36	0.000
B vs E	32.88 vs51.71	0.000	32.88 vs51.71	0.000	170.72 vs922.08	0.000
C vs D	102.82 vs50.43	0.000	32.88 vs50.43	0.000	441.59 vs810.36	0.000
C vs E	102.82 vs 102.82	0.000	32.88 vs51.71	0.000	441.59 vs922.08	0.000
D vs E	50.43 vs 51.71	0.462	50.43 vs51.71	0.000	810.36 vs922.08	0.000

Table 3 One way analysis of variance of clinical parameters, salivary , GCF and serum elastase among different category of pack years of smoking periodontitis.

Pack Years		**PI**	**GBI**	**PPD**	**CAL**	**Salivary Elastase**	**GCF Elastase**	**Serum Elastase**
Upto 5 Years	Mean	2.14	72.28	5.97	5.47	0.52	0.47	7.90
	S.D.	0.50	3.88	1.03	0.63	0.05	0.05	0.22
5 – 10 Years	Mean	2.18	70.50	5.79	5.35	0.51	0.59	8.78
	S.D.	0.48	3.90	0.80	0.97	0.06	0.14	0.76
Above 10 Years	Mean	2.13	70.92	5.60	5.66	0.52	0.61	8.95
	S.D.	0.65	4.73	0.82	1.03	0.04	0.10	0.28
Total	Mean	2.17	70.85	5.79	5.41	0.51	0.57	8.66
	S.D.	0.50	3.98	0.83	0.92	0.05	0.13	0.73
'F' – value		0.032	0.643	0.357	0.353	0.336	2.987*	6.602**
'p' – value		0.969	0.530	0.702	0.704	0.717	0.050	0.003

Table 4 Correlation analysis between PI, GBI, PPD, CAL, Salivary Elastase, GCF Elastase, Serum Elastase and Pack Years

		PI	GBI	PPD	CAL	Salivary elastase	GCF elastase	Serum elasatse	Pack yrs
PI	Pearson Correlation	1							
	Sig. (2-tailed)								
GBI	Pearson Correlation	.140	1						
	Sig. (2-tailed)	.333							
PPD	Pearson Correlation	.202	-.179	1					
	Sig. (2-tailed)	.160	.215						
CAL	Pearson Correlation	.026	-.106	-.252	1				
	Sig. (2-tailed)	.859	.464	.077					
Salivary Elastase	Pearson Correlation	-.427**	-.193	-.123	.211	1			
	Sig. (2-tailed)	.002	.178	.396	.141				
GCF Elastase	Pearson Correlation	-.163	-.008	-.024	-.350*	.097	1		
	Sig. (2-tailed)	.258	.954	.868	.013	.502			
Serum Elaste	Pearson Correlation	-.154	-.104	-.139	-.026	.018	.536**	1	
	Sig. (2-tailed)	.286	.471	.335	.857	.899	.000		
Pack Years	Pearson Correlation	-.002	-.102	-.122	.053	.021	.287*	.410**	1
	Sig. (2-tailed)	.986	.480	.398	.714	.885	.043	.003	

** Correlation was significant at the 0.01 level (2-tailed).
* Correlation was significant at the 0.05 level (2-tailed).

This was well documented by **Aaron** *et al*15 where they demonstrated increased elastase activity in smokers lungs fluid due to increased recruitment of macrophages and neutrophils to smokers lungs and decreased activity of lung elastase inhibitors in smokers.

In GCF, since the collection is from site specific areas and deep pockets, there was increased elastase activity of smokers which highlighted a dose-dependent relationship.

These results reflect the positive association between the pack years with that of GCF & serum elastase activity.

Whereas In saliva, due to migration of neutrophils in blood and also lung tissues in smokers, along with contamination during collection, it showed a non- reflective activity.

CONCLUSION

Smoking shows a profound effect on the periodontal tissues and causes numerous changes in the tissues which maybe reflected clinically or masked due the deleterious effects of smoke constituents. Elastase proves to be a reliable marker not only in diagnosing the periodontal disease severity, locally and systemically. But also Furthermore helps in future prediction and management of the disease.

Limitations of the study

- In our study, Former/ ex-smokers were not included, if it has been included, more predictable association between elastase activity and periodontal destruction would have been determined.
- Periodontitis severity was not categorised as mild, moderate and severe form.

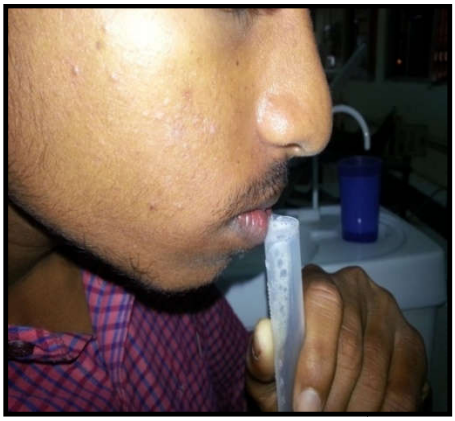

Fig 1 Collection of saliva samples

Fig 2 Collection of GCF samples

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