Effectiveness of Periodontal intervention on the levels of N Terminal Pro Brain Natriuretic Peptide in Gingival Crevicular Fluid and Serum in Chronic Periodontitis patients

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Abstract

Background- N-terminal-pro-brain natriuretic peptide (NT-proBNP) is an inactive hormone which is seen during inflammation and is a known biomarker of cardiovascular disease (CVD). Evidence suggests that periodontitis has a bidirectional relationship with CVD and NTProBNP has a potential role in periodontal disease. However, there is no evidence on the impact of Non-surgical periodontal therapy (NSPT) on the levels of NTProBNP in GCF and serum in patients with chronic periodontitis. Hence, the aim of this study wa**s** to compare the levels of NT-proBNP in GCF and serum in patients with Chronic generalized periodontitis before and after NSPT after 6 weeks.

Methods: An interventional study was carried out in which GCF and serum samples was collected in 19 patients with chronic generalized periodontitis prior and post NSPT 45 days and the cumulative or reduction in values of NTProBNP in both parameters; GCF and serum was assessed. All subjects underwent nonsurgical periodontal therapy (NSPT) including complete SRP and subgingival debridement. NTProBNP levels in GCF and serum were determined by Enzyme Linked Immunosorbent Assay (ELISA).

Results: The concentrations of NTProBNP were significantly reduced in GCF and serum after NSPT. Statistically significant difference of BNP concentration between pre and post groups in GCF were apparent (p <0.0001) whereas statistically non-significant results in NTProBNP serum levels when compared at baseline to post-operative state with the mean of 61.77 (22.6 SD) pre operatively and 72.67 (51.86 SD) post operatively. *(p-value = 0.0007)* was observed.

Conclusion: Significant reduction of NTProBNP concentrations in GCF and serum in patients with chronic generalized periodontitis subjected to non-surgical periodontal therapy was observed. This may account for a significant relation between periodontal disease, bacteremia and cardiovascular disease.

Background

Periodontitis is a multifactorial inflammatory disease caused by interactions between periodontal microflora and host immune response. Perpetuating periodontal destruction is an important sequela of periodontitis that can lead to the subsequent loss of teeth. The expression of the disease results from the interaction of host defense mechanisms, microbial agents, environmental, and genetic factors.

Traditional clinical diagnosis of periodontal disease cannot reliably identify the sites of ongoing periodontal destruction and does not provide information about the patient's susceptibility to disease, whether the disease is progressing or not, or in remission, or the response to treatment will be positive or negative. Although specific microorganisms are implicated in periodontitis, many other aspects of tissue changes are known to negatively alter periodontal status. Based on this concept, serum, gingival tissue fluid, saliva, and tissue biopsy samples have now been investigated for periodontal adversities and their association with markers associated with systemic complications

GCF contains a variety of biochemical factors that may be used as biomarkers for the diagnosis or prognosis of periodontal tissue biological conditions in health and disease. GCF, both stimulated and unstimulated, can be obtained non-invasively from various oral sites and has been shown to be a reliable predictor of disease activity. Due to the increased activity of the disease, many proteins and enzymes in GCF increase. Means obtained for each standard marker activity are observed at GCF, which is 20-fold higher than serum.

The prime objective of scaling and root planning is to restore gingival health by completely removing the tooth surface elements that provoke the gingival inflammation, to provide a biologically acceptable root surface and to facilitate oral hygiene.

Clinical and radiographic assessment is used as traditional measures for diagnosis of periodontal diseases. These traditional measures give only information about the existing disease but limited to predict in early diagnosis of disease. Therefore, advances in oral and periodontal disease diagnostic research are moving toward methods, whereby, periodontal risk can be identified and quantified by objective measures like biomarkers. One of these biomarkers that can be detected in several inflammatory conditions is NTProBNP.

Brain natriuretic peptide (BNP) is a 32 amino acid peptide that is released primarily from the ventricular myocardium in response to increased myocardial wall tension. BNP is produced as a prohormone that is cleaved into two peptides: the active hormone BNP; and a biologically inactive N-terminal proBNP (NTproBNP). Because of its longer half-life compared to active BNP, it has been suggested that serum NTproBNP may be a viable biomarker for CVD.[1] This peptide is also associated with an increased risk of CV events, cerebral ischemia, and all causes that increases the mortality rate.

In a previous study, patients with periodontitis showed significantly higher NT-proBNP serum levels than subjects without periodontitis. Serum NT-proBNP levels increased as periodontal disease destruction progressed[2]. Recent studies have shown an association between periodontitis and elevated serum NTproBNP levels.[1]

To the best of our knowledge, the relationship between the gingival crevicular fluid and serum levels of NTProBNP in chronic periodontitis and the effect of SRP on their levels have not been explored. Hence, this study aims to compare the levels of NT-proBNP in serum and GCF in patients with Chronic generalized periodontitis before and after SRP.

Methods

The study was conducted at Department of Periodontology, Faculty of Dental Sciences, Ramaiah University of Applied Sciences in collaboration with Department of Microbiology, Ramaiah Medical College and Hospital, Bangalore. Study period was January 2020 to November 2021.

This study was an interventional study in which serum and GCF samples were collected from 19 patients with chronic periodontitis. Around 45 days of SRP and cumulative or decreased NTProBNP levels for both parameters were collected. GCF and serum were evaluated. Subjects referred to the Department of Periodontology who met the selection criteria were evaluated and included in the study. 19 patients who met the selection criteria were considered sample size for this study. GCF and serum samples were taken before and after SRP. All subjects received non-surgical periodontal treatment (NSPT), which includes complete SRP and subgingival debridement.

Inclusion criteria were: Patients within the age 25-50 years, systemically healthy patient, subjects with ≥18 completely erupted teeth, subjects with presence of Bleeding on Probing, probing pocket depth ≥ 5mm, clinical attachment level ≥ 6mm. The Exclusion criteria was: Atherosclerotic vascular disease (i.e., CVD, stroke, and peripheral artery disease), Immunological disorders, arthritis/osteoporosis, history of periodontal intervention within the last 6 months, anti-inflammatory and nonsteroidal anti-inflammatory therapy within 3 months prior to periodontal assessment, pregnancy or lactation.

The research was performed in *accordance* with the Declaration of Helsinki of the World Medical Association (2008) and was *approved* by the Ethics Committee for Human Trials of M.S. Ramaiah University of Applied Sciences with reference no: *EC-2020/PG/13. Informed consent* was obtained from each patient or their relatives after full explanation of the periodontal examination, GCF and blood sample extraction.

The sample size has been estimated using the GPower software v. 3.1.9.4

Considering the effect size to be measured (dz) at 50%, power of the study at 80% and the margin of the error at 10%, the total sample size needed was 19.

COLLECTION OF SERUM AND GCF NT-PROBNP BEFORE SRP:

NT-ProBNP in serum and GCF levels was assessed in patients with periodontitis before scaling and root planing. Complete periodontal examination was performed in all subjects. (Figure 1)

Blood samples was obtained in the morning (Figure 3). This is important because the human body is subjected to variations depending on the time of day, due to this variability of parameters during the day the values are observed to alter which is reflected in laboratory results[3] and both the Canadian and U.S. hematology guidelines endorse this view. Although still in debate, fasting before drawing blood is not recommended because the body starts to use its own protein, especially with a small supply of fat. This can lead to glucose levels being too low and even to increased amounts of ketone compounds or a reduction in iron and hemoglobin levels. Subjects should be seated and relaxed and asked not to be anxious as it may cause the body to stimulate and release adrenaline. Therefore, a blood test preceded by physical effort or anxiousness will manifest itself in the form of altered blood serum levels[4]. Briefly, 2 mL

of venous blood was collected from the antecubital fossa by venipuncture using a 20-gauge needle with a 2 mL syringe. Blood samples was allowed to clot at room temperature (Figure 4) and, after 1 hour, serum was separated from blood by centrifugation (Figure 6) and 0.5 mL of extracted serum was immediately transferred to 1.5-mL aliquots. Each aliquot was stored at −80°C until required for analysis.

The GCF samples from deepest probing depth was collected for NTProBNP assessment (Figure 2). The GCF samples from all the patients was collected after 24 h following baseline examination to avoid contamination of the sample with blood. GCF samples was obtained from the sites with deepest probing depth. Supragingival plaque of the intended tooth was removed with piezoelectric ultrasonic scaler before sampling (Figure 7). The tooth was dried prior to obtaining the sample. The GCF collection was done using micropipettes with proper isolation of the site with cotton rolls.

Ultrasonic scaling and root planing procedure was performed (Figure 5) and the extent was re evaluated for any local factors post operatively. (Figure 7)

COLLECTION OF SERUM AND GCF NT-PROBNP AFTER SRP:

NTProBNP in serum and GCF levels was assessed in patients with chronic generalized periodontitis after scaling and root planing after 45 days.

Complete periodontal examination was performed in all subjects and all the clinical parameters was assessed. The procedure for assessing the serum and GCF levels was repeated, blood samples was obtained in the morning. Briefly, 2 mL of venous blood was collected from the antecubital fossa by venipuncture using a 20-gauge needle with a 2 mL syringe. Blood samples was allowed to clot at room temperature and, after 1 hour, serum is separated from blood by centrifugation and 0.5 mL of extracted serum was immediately transferred to 1.5-mL aliquots. Each aliquot was stored at −80°C until required for analysis.

The GCF samples from deepest probing depth was collected for NTProBNP assessment. The GCF samples from all the patients was collected after 24 h following baseline examination to avoid contamination of the sample with blood. GCF samples was obtained from the sites with deepest probing depth (Figure 8). The tooth was dried prior to obtaining the sample. The GCF collection was done using micropipettes with proper isolation of the site with cotton rolls.

COMPARING THE LEVELS OF NT-PROBNP IN SERUM AND GCF:

The levels of NTProBNP in serum and GCF levels in patients before and after scaling and root planing was compared and the values obtained will determine whether scaling and root planing have associated

effect on the levels of serum and GCF NTProBNP.

Standard Preparation:

Reconstitution of original BNP standard with 1 ml of sample diluent was done. Standards were kept for 15 mins with gentle agitation before making further dilutions. Additional standards were prepared by serially diluting the standard stock solutions as per given in the table below:

Table 1

indicates the standard values or concentrates obtained from the serum and GCF samples of BNP, which procures a standard curve to as the measure the values in the samples.

STANDARD CONC.	STANDARD VIAL	DILUTIONS
2000 pg/ml	Standard no. 8	Re constitute with 1ml sample diluent.
1000 pg/ml	Standard no. 7	300 ul Standard no. 8 + 300ul sample diluent.
500 pg/ml	Standard no. 6	300 ul Standard no. 7 + 300ul sample diluent.
250 pg/ml	Standard no. 5	300 ul Standard no. 6 + 300ul sample diluent.
125 pg/ml	Standard no. 4	300 ul Standard no. 5 + 300ul sample diluent.
62.5 pg/ml	Standard no. 3	300 ul Standard no. 4 + 300ul sample diluent.
31.25 pg/ml	Standard no. 2	300 ul Standard no. 3 + 300ul sample diluent.
0 pg/ml	Standard no. 1	300ul sample diluent.

NTProBNP ELISA QUANTITATIVE ASSAY PROCEDURE:

1. Bring all the reagents to room temperature before use. (Figure 9)
2. Pipette standards 1-8 samples – about 100 ul. (Figure 10)
3. Incubate for 90 minutes.
4. Wash 1x wash buffer Decant, 4 x 300 ul
5. Pipette biotinylated anti-BNP 100ul (Figure 11)
6. Incubate 60 minutes (37 degree C)
7. Wash 1x wash buffer Decant, 4 x 300 ul
8. Pipette streptavidin: HRP conjugate 100ul
9. Incubate 30 minutes
10. Wash 1x wash buffer Decant, 4 x 300 ul

11. Pipette TMB substrate 90 ul (Figure 12)
12. Incubate in dark 10 minutes.
13. Pipette stop solution 50ul (Figure 14)
14. Measure 450 within 15 minutes.

(Figure 17) Standard curve generated using the obtained standard concentration (Table 2) to measure the concentrations of NTProBNP in GCF and serum.

Table 2

The values obtained following the processing the standards are given below:

STANDARD CONC.	values	Concentration	CONC. OF NT-ProBNP
S1	0.066	0	0
S2	0.122	31.25	60.011
S3	0.165	62.5	65.485
S4	0.304	125	86.836
S5	0.647	250	174.231
S6	1.061	500	403.787
S7	1.498	1000	980.521
S8	1.977	2000	2952.945

NTProBNP level detection was performed by enzyme immunoassay (ELISA) in an ELISA plate analyzer (Figure 13). AntiBNP antibody was pre-coated in 96-well plates and biotin-conjugated anti-BNP antibody was used as detection antibody. Then, the calibrator, test sample and biotin-conjugated detection antibody were added to the well and washed with wash buffer. Horseradish streptavidin peroxidase (HRP) was added and unbound conjugates were washed with wash buffer. Tetramethylbenzidine (TMB) substrate will be used to visualize the HRP enzymatic reaction. The TMB will be catalyzed by HRP to give a blue colored product which will turn yellow after the addition of the acid solution stops. The density of the yolk will be proportional to the amount of NTProBNP sample collected in the plate. The optical density absorbance (O.D.) reading should be recorded at 450 nm in a microplate reader (Figure 15) and then the NTProBNP concentration should be calculated (Figure 16). Demographic, clinical and historical information for certain diseases will also be recorded

STATISTICAL ANALYSIS:

Statistical Package for Social Sciences [SPSS] for Windows Version 22.0 Released 2013. Armonk, NY: IBM Corp., will be used to perform statistical analyses.

Descriptive Statistics:

Descriptive analysis of all the explanatory and outcome parameters will be done using mean and standard deviation for quantitative variables, frequency and proportions for categorical variables.

Inferential Statistics:

Student Paired t Test will be used to compare the mean values of clinical parameters and NTProBNP levels (GCF & Serum) levels before and after scaling and root planing periods. Pearson correlation test will be used to assess the relationship between clinical parameters and NT-ProBNP levels (GCF & Serum) levels at before and after scaling and root planing periods. Stepwise Multiple linear regression analysis will be performed to predict the variation in the NT-ProBNP levels (GCF & Serum) levels in context to clinical parameters before and after scaling and root planing periods. The level of significance [P-Value] will be set at $P<0.05$ and any other relevant test, if found appropriate during the time of data analysis will be dealt accordingly.

Processing the Elisa kits and estimating the contorted BNP values in GCF and serum was done in Department of Microbiology, Ramaiah Medical College, Bangalore.

Results

In total 20 subjects were recruited and treated, and their GCF and serum samples were collected prior and post SRP. The sample population comprised of 11 females and 9 males (Fig. 18) and were in the age range of 18–50 years (Fig. 19). There was no significant difference between the two groups regarding age and gender.

The purpose of this intervention study was to estimate GCF and serum NTProBNP levels 45 days before and after the intervention. In this study, non-surgical periodontal scaling and root planing were selected as treatment modality and NTProBNP levels were evaluated 45 days after NSPT.

(Table 3) signifies the pre- and post-operative serum values. Although few subjects showed reduction in concentrations of serum NTProBNP, however, upon estimation it was found that, in 36.8% of subject's (n = 7) serum NTProBNP exhibited higher values even after non-surgical periodontal therapy. Nevertheless, upon clinical evaluation of periodontal parameters, periodontal status of the subjects was improved Hence, serum concentration values were not statistically significant. ($p > 0.0005$)

Table 3
Pre-operative and Post-operative BNP serum values

BNP SERUM SAMPLES		
SAMPLE ID	PRE-OPERATIVE	POST OPERATIVE
D129	56.58	56.81
D584	56.69	56.47
D570	56.58	55.67
D569	57.27	55.44
D557	56.01	55.33
D124	55.22	55.33
D048	55.78	52.91
D045	57.27	56.24
D541	56.24	59.53
D397	56.35	58.45
D099	57.74	279.69
D569	57.62	56.35
D875	59.04	56.01
D868	57.39	57.04
D587	55.89	57.74
D035	56.35	79.74
D577	55.44	57.98
D453	158.05	133.81
D273	58.21	55.78

(Table 4) signifies the pre- and post-operative NTProBNP GCF values. The table indicate that there is a significant reduction in GCF post scaling and root planing. Subjects showed drastic reduction in GCF-BNP values whereas 36.8% subjects (n = 7) showed slight increased concentrations in GCF levels. Clinical evaluation of periodontal parameters showed that periodontal status of the subjects was improved. Hence, GCF concentration values were statistically significant. ($p < 0.0001$)

Table 4
Pre-operative and Post-operative BNP GCF values

BNP GCF SAMPLES		
SAMPLE ID	PRE- OPERATIVE	POST OPERATIVE
D129	59.16	56.81
D584	76.26	58.45
D570	56.81	58.09
D569	58.57	59.40
D557	69.18	58.21
D124	56.81	57.21
D048	57.51	57.16
D045	58.21	50.5
D541	57.86	58.21
D397	56.69	58.21
D099	57.27	62.50
D569	57.16	57.98
D875	58.92	55.11
D868	59.40	50.81
D587	58.09	49.48
D035	67.37	50.40
D577	56.58	50.09
D453	57.62	50.29
D273	685.93	49.79

There is a significant increase in NTProBNP serum levels when compared at baseline to post-operative state with the mean of 61.77 (22.6 SD) pre operatively and 72.67 (51.86 SD) post operatively. *(p-value = 0.0007)* (Table 5)

Table 5
Statistical and Numerical Analysis of the values obtained for Serum NTProBNP

SERUM	Mean (+/- SD)	*p*- value
Pre-operative	61.77 (+/- 22.6)	0.0007
Post-operative	72.67 (+/- 51.86)	

Table 6
Statistical and Numerical Analysis of the values obtained for GCF NTProBNP

SERUM	Mean (+/- SD)	*p*- value
Pre-operative	90.11 (+/- 140.11)	< 0.0001
Post-operative	54.93 (+/- 4.23)	

(Table6) shows that there is a significant decrease in GCF levels of NTProBNP when compared at baseline to post-operative state, this indicates that GCF is an absolute virtuous indicator for assessing NTProBNP levels in patients with periodontitis.

Analysis was done by Fischer's analysis test because the significance of the deviation from p-value can be calculated exactly, rather than relying on an approximation that becomes exact in the limit as the sample size grows to infinity, as with many statistical tests. Significant difference of BNP concentration between pre and post groups in GCF were apparent and the mean concentration in the pre-SRP group was high when compared to Post-SRP group which had evidently reduced post intervention. ($p < 0.0001$)

Statistically non-significant difference of BNP concentration between Pre and post groups in serum were observed and similarly the mean concentration of BNP in serum was seen to be increased post intervention as compared pre intervention. ($p = 0.0007$)

Discussion

Chronic periodontitis is a common condition, affecting 45.9% of the US adult population aged 30 years and older[5]. Chronic periodontitis causes loss of connective tissue that supports teeth and alveolar bone, which, if left untreated, is a major cause of tooth loss in adults[6]. According to the case definitions of the Centers for Disease Control and Prevention and the American Academy of Periodontology, the estimated prevalence of early/moderate and severe periodontitis is 37.1 and 8.9% in adults, respectively in the United States [5].

This study was conducted on 19 subjects having periodontitis and NTProBNP level estimation was done in GCF and serum on the same. Patients suffering from any acute systemic inflammatory conditions

were excluded in this study because levels of NTProBNP may be affected as BNP (inactive) is an acute phase reactant protein[7], where its serum concentrations rise significantly during acute inflammation.

In addition, since diabetes has a great impact on periodontal disease and treatment, we excluded patients with such systemic diseases. NTProBNP also has an indirect effect on pancreatic insulin-producing β-cells through the accumulation of intracellular iron. Patients with cardiovascular disease, neurodegenerative disease, chronic infections (eg, tuberculosis and degenerative disease), cancer, and liver disease were also excluded as these diseases impair the onset, progression, and outcome of periodontal disease and may affect the NTProBNP concentration levels.

This study shows that patients with periodontitis have higher serum and GCF NT-proBNP levels. Moreover, as periodontal disease progresses, serum and GCF NT-proBNP levels

increases which reduce adequately after SRP. BNP is a vital cardiovascular biomarker which is actively detected in periodontal diseases, SRP aids in the improvement in clinical parameters, which may be due to resolution of the inflammatory response and the cessation of periodontal destruction which makes a direct impact on this biomarker and reduces its potential pathogenicity and leads to their lowered concentrations in GCF and serum. This could be explained by invasion of periodontal pathogen into vascular endothelial cells in order to evade the immune cells. Endothelial invasion by P. gingivalis is facilitated by hemagglutinin A, hemagglutinin B, and fimbriae A. Fimbriae A increases expression of different adhesion molecules on the endothelial cells of blood vessels and expression of inflammatory mediators such as IL-6, IL-8, and cyclooxygenase-2. In fact, LPS and Fimbriae A could coordinate in the proinflammatory stimulation of arterial endothelium by P. gingivalis. Aggregatebacter actinomycetemcomitans also up-regulate the formation of adhesion molecules, however, in lesser amount. This suggests that CVD and periodontal disease have a bidirectional relationship and periodontal intervention has vital influence on periodontal and CVD parameters which may perhaps decrease the risk of CVD.

In this study, during the estimation of NTProBNP concentrations in GCF we found that there is substantial decrease in the levels post SRP when compared at the baseline. Significant difference of NTProBNP concentration between pre and post state in GCF were apparent and the mean concentration in the pre-SRP group was high when compared to Post-SRP group which had evidently reduced post intervention. ($p < 0.0001$). however, when concentration of NTProBNP in serum were estimated few subjects did not show significant reduction. Upon estimation it was found that, the serum levels in NTProBNP with subjects having periodontitis revealed increased values. However, upon clinical evaluation of periodontal parameters, periodontal status of the subjects was improved. This would be because of elimination of local factors and patient education along with maintenance. Hence, serum concentration values are were not statistically significant. ($p = 0.0007$)

In accordance with the present study, Mahendra Mohan et al., assessed C- Reactive Protein levels after SRP evaluated at baseline, 1 month and 45 days in patients with Diabetes Mellitus and Chronic

Periodontitis (DM-CP) and non-diabetic chronic periodontitis (NDM-CP) patients and showed similar results suggesting that the CRP levels in both GCF and serum were higher in DM-CP patients than in NDM-CP patients[8]. Although there was a significant improvement in both the groups, greater improvement was observed in both GCF and serum samples of T2DM-CP patients. Our results are consistent with the results of this study showing a significant reduction in GCF and serum NTProBNP levels after SRP compared to baseline 45 days. Both showed a decrease, but a greater decrease in BNP concentration was observed with GCF compared to serum.

However, Paschalina Goutoudi et al. concluded that periodontal therapy reduced IL8 levels in GCF[9]. This finding is consistent with our study where NTProBNP levels decreased in GCF after SRP. However, there is a result contrary to our study that the concentration of NTProBNP before and after periodontal intervention is not statistically significant. A close association was observed between periodontal destruction and NTProBNP levels. Patients with periodontitis initially presented with higher periodontal inflammation with high NTProBNP levels while when SRP was performed, these values decreased after 45 days, NTProBNP levels decreased significantly in GCF and serum, because there is an inflammatory response and stops the destruction of periodontal disease affecting NTProBNP, thereby reducing their levels.

Samah H. Elmeadawy et al., have shown that non-surgical periodontal treatment significantly reduces serum VCAM1 levels. The VCAM1 protein mediates the adhesion of lymphocytes, monocytes, eosinophils, and basophils to the vascular endothelium and may be involved in the development of atherosclerosis and rheumatoid arthritis[10]. Another study by Cui D et al., have shown reduced VCAM1 expression and macrophage / monocyte infiltration in the vascular wall of mice treated with non-surgical periodontal treatment[11]. Both studies were consistent with our study that GCF NTProBNP levels decreased 45 days after receiving SRP.

Deng et al., however reported NSPT had no significant effect on CRP, total cholesterol, LDL-C and triglycerides[12]. Also, Zang et al., concluded NSPT was associated with carotid atherosclerosis but statistical heterogeneity was substantial, hence the results were obsolete and trajected towards showing NSPT having no effects on CVD biomarkers[13]. These studies showed contradictory results when compared to our study. Our study showed substantial homogeneity in results where NSPT has an effect on the NTProBNP levels in GCF and serum in patients with periodontitis.

Milli et al.,2015 correlated the levels of sCD40 L and MCP-1 in serum and gingival crevicular fluid (GCF) of patients with chronic periodontitis before after SRP. sCD40 L and MCP-1 are the acute precipitators of CVD in subjects with periodontitis, concluded that a positive correlation observed between sCD40 L and MCP-1 levels and detected reduced levels of GCF and serum after SRP which suggest this phenomenon as one of the pathways that may lead to the propagation of cardiovascular events in patients with periodontal disease[14]. This is in accordance with our study as NTProBNP levels are reduced post SRP which shows a positive co relation between CVD and periodontitis and may prevent the risk of CVD.

For this study, SRP was found to significantly reduce NTProBNP levels in GCF in patients with chronic periodontitis compared to baseline values. This decrease in NTProBNP levels at the end of the study can be explained by the effect of SRP on local factor removal. Therefore, it can reduce the inflammatory response and, as a result, the mediators that stimulates the production in the acute phase protein.

We were unable to compare the results of the present study on the effect of SRP on serum or GCF levels of NTProBNP in patients with chronic periodontitis with other studies because to our knowledge this is the first study to evaluate NTProBNP levels in GCF and serum in patients with periodontitis before and after SRP.

The Significant increase in the concentration levels of NTproBNP in serum may perhaps be due to following reasons:

1. Time Factor: NTProBNP, is a cardiac biomarker which is detected in patients with periodontitis. The reduction of the levels in serum requires supplemental time in the blood stream to get stabilize. Also, evidences regarding CVD biomarkers suggest the recall period as 3–6 months for re-evaluation.
2. Periodontal Invention: Evidence by *Alka S. Waghmare et al., 2014* concluded that bacteraemia frequently occurs immediately after SRP[15]. Similarly, in our study SRP may have led to bacteraemia and therefore had aggravated the levels of serum NTProBNP for a brief time.

This study had a number of shortcomings. Because of the inclusion criteria of never-smokers and individuals without diabetes, total number of patients to be included in the study was limited. Blinding was done only at the statistician level. Thus, multilevel blinding can be incorporated in the future studies. The long-term effect of NSPT on the improvement of cardiac status can be further ascertained in future studies.

Conclusion

Till date and to best of our knowledge this is the first study that aims to compare the levels of NT-proBNP in serum and GCF in patients with Chronic generalized periodontitis before and after SRP. Even with the limitations of this study, we can conclude that non-surgical periodontal therapy has a reducing effect on the serum and GCF NTProBNP levels in chronic periodontitis patients. In addition, serum and GCF BNP levels represent a potential biomarker of the chronic periodontitis disease and may indicate non-surgical periodontal therapy may avoid the risk of CVD events by reducing the systemic inflammation caused by local factors. Hence, it can be concluded that NSPT reduces the NTProBNP concentrations in patients with chronic periodontitis and may evade the future risk of CVD.

Declarations

Ethics approval and consent to participate:

- The study protocol is approved by the University Ethics Committee for Human Trials of M S Ramaiah University of Applied Sciences (Ref no. EC-2020/PG/13). ethics committee.
- Written informed consent has been obtained from the participants.

Consent for publication:

Obtained from the study participants.

Availability of data and materials:

All data generated or analyzed during this study are included in this published article (and its supplementary information files).

Competing interests:

None

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Authors Contribution:

- IF: Clinical investigator; funding; data collection; manuscript preparation
- BS: Designing of the study; clinical investigator; data analysis
- UY: Literature search; manuscript review
- SFK: Study concept; manuscript preparation, editing and review
- MN: Manuscript preparation and review

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Figures

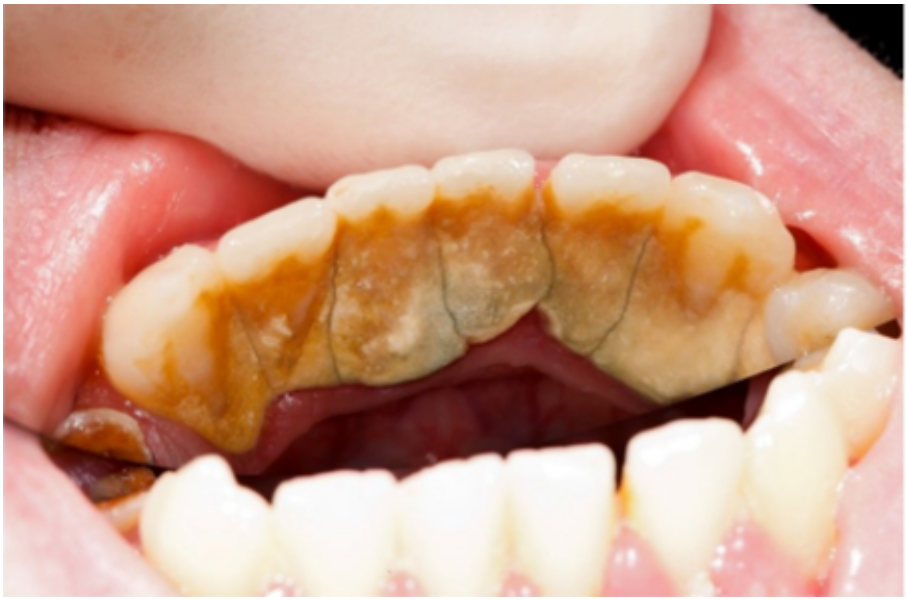

Figure 1

Pre-operative SRP

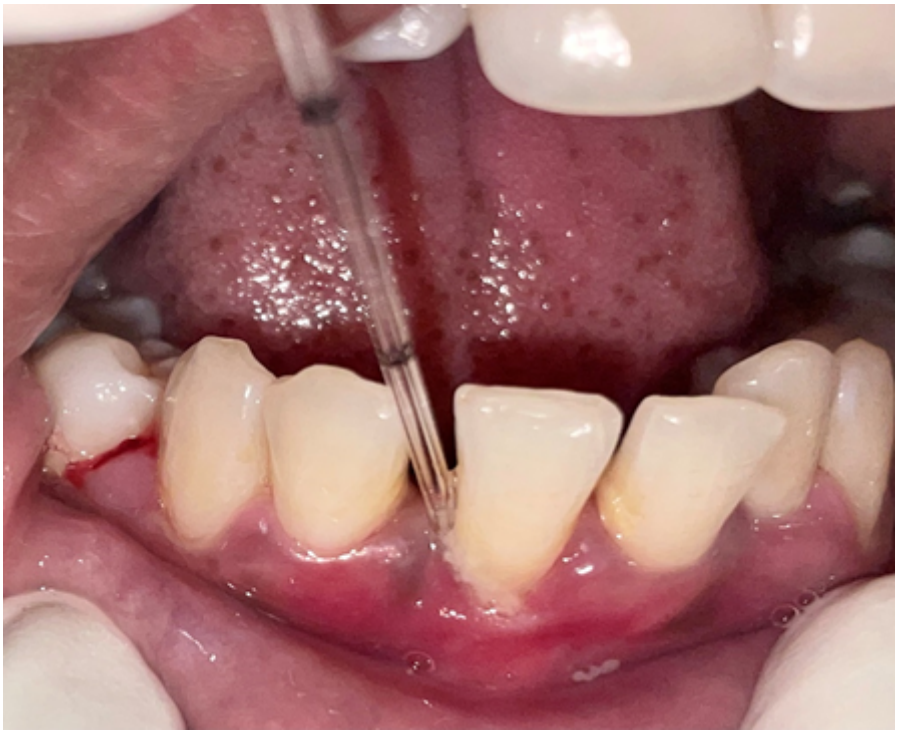

Figure 2

Collection of GCF samples.

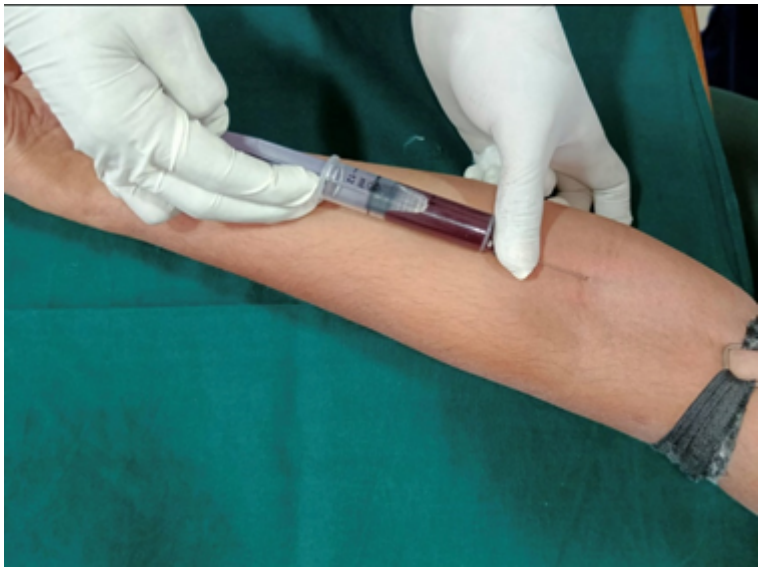

Figure 3

Collection of blood sample from Antecubital vein.

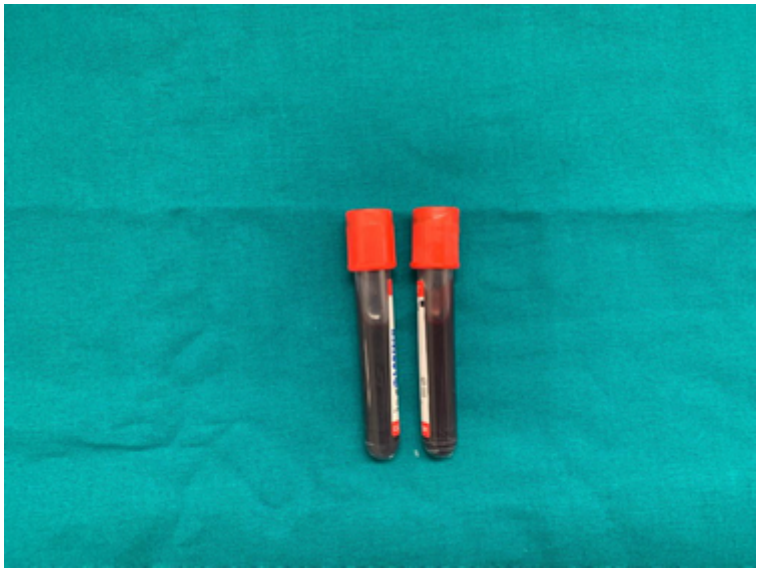

Figure 4

Blood sample before centrifuging.

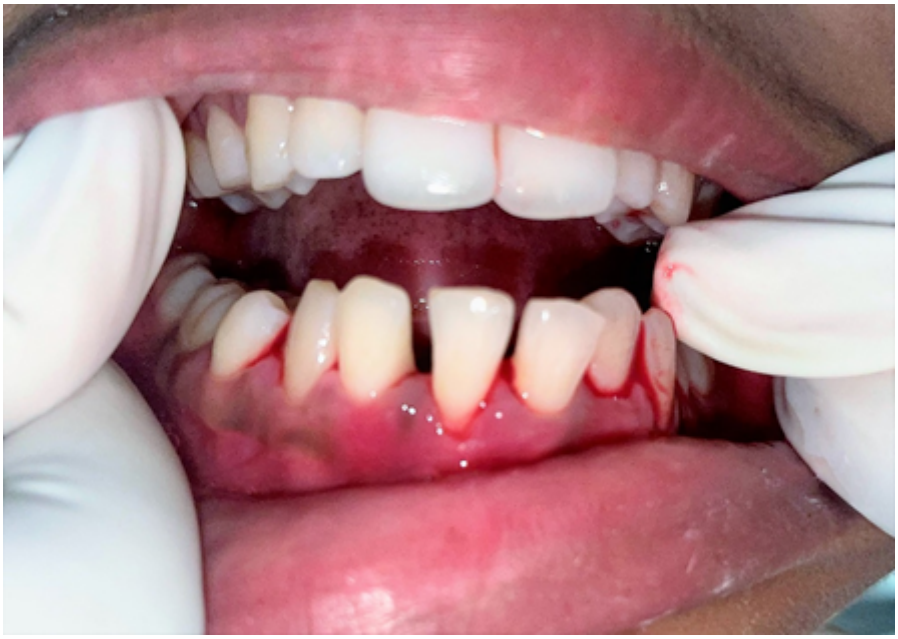

Figure 5

Intra operative SRP

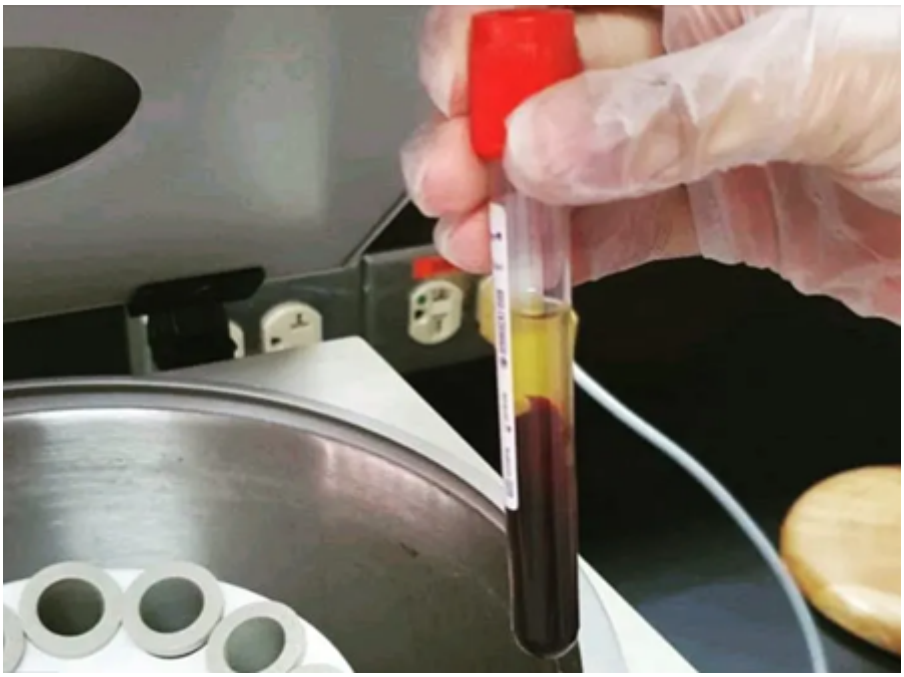

Figure 6

Serum sample obtained after centrifugation.

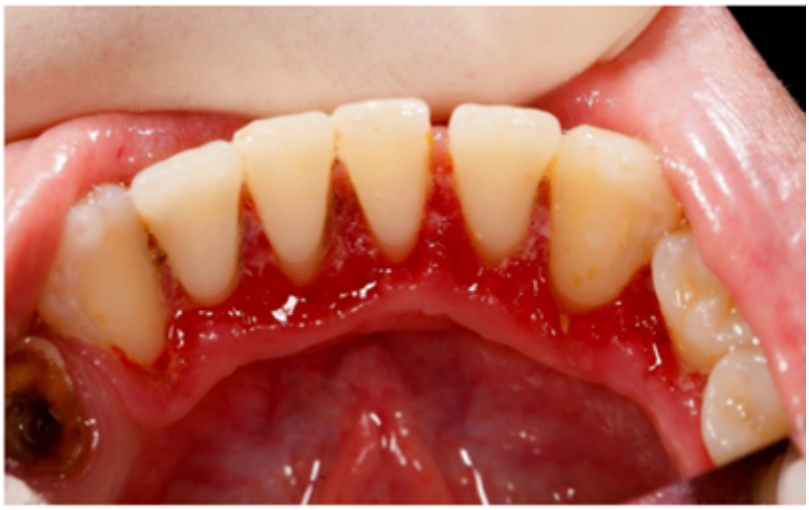

Figure 7

Post-operative SRP

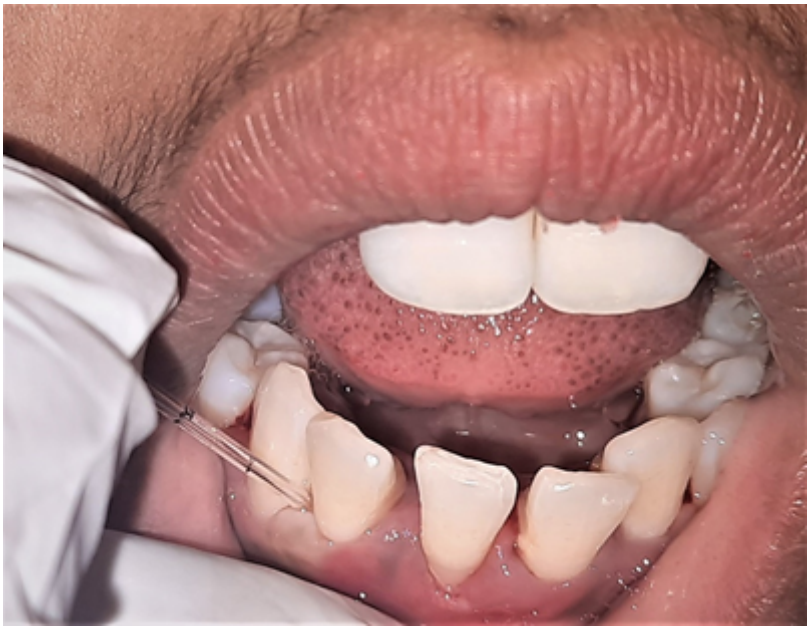

Figure 8

Intra operative GCF collection

Figure 9

NTProBNP enzyme-linked immunosorbent assay kit. California, USA

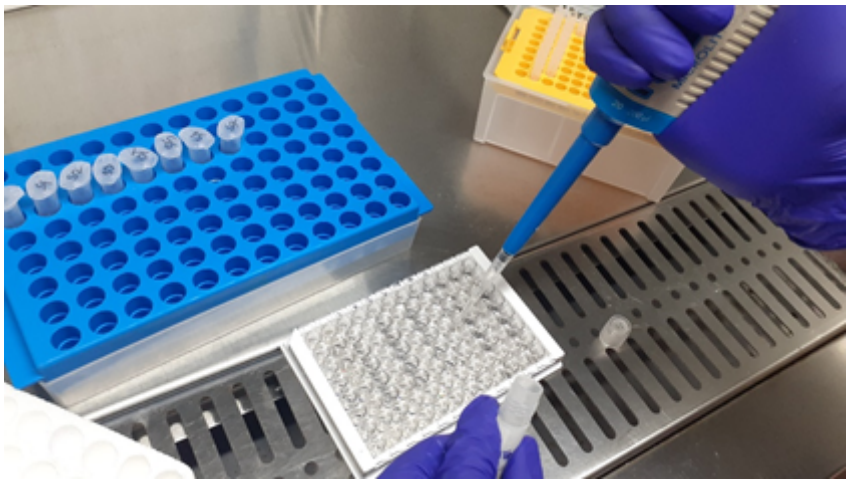

Figure 10

(left) 8 standards in vials. (Right) adding serum and GCF samples in the microtiter plate

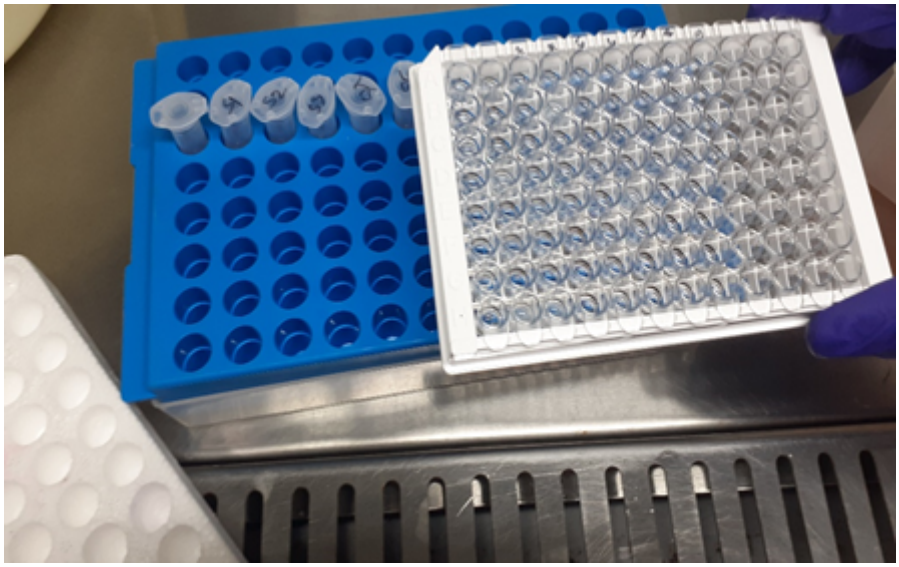

Figure 11

antibody coated microtiter wells after adding biotinylated BNP antibody working solution and after wash buffer.

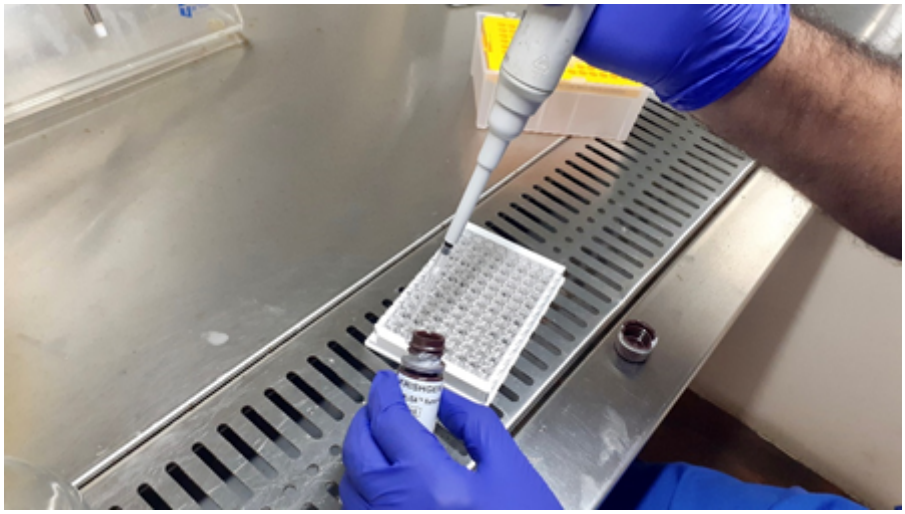

Figure 12

Retrieval and pipetting of tetramethylbenzidine reagent

Figure 13

ELISA plate analyzer

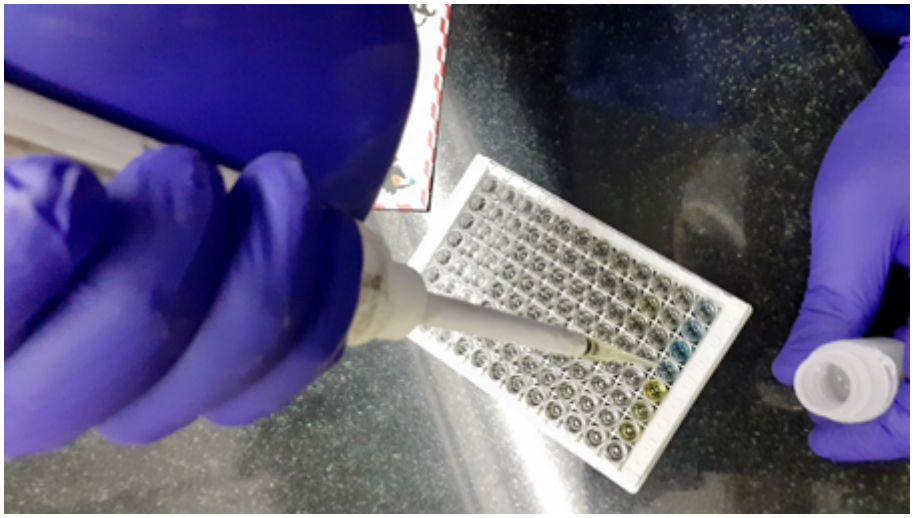

Figure 14

Pipetting Stop solution- blue to yellow

Figure 15

microtiter well plate kept in enzyme-linked immunosorbent assay reader

Figure 16

Estimated values obtained.

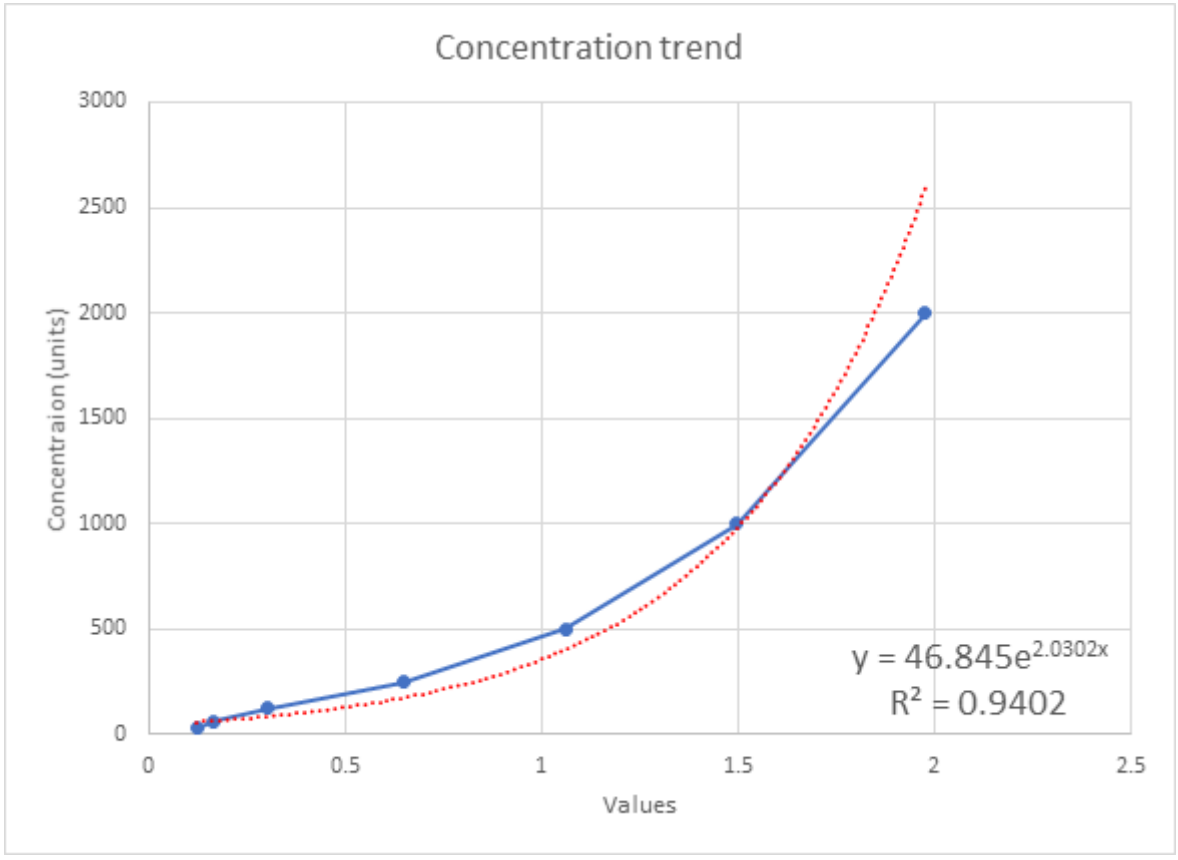

Figure 17

Standard curve for concentration trend.

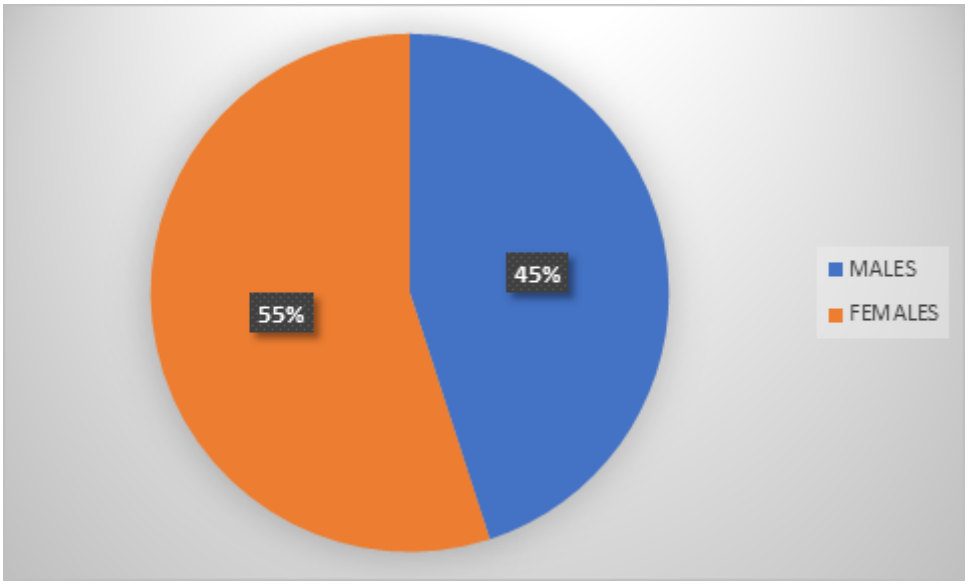

Figure 18

graphical presentation of subjects' gender distribution taken for collection GCF and serum samples

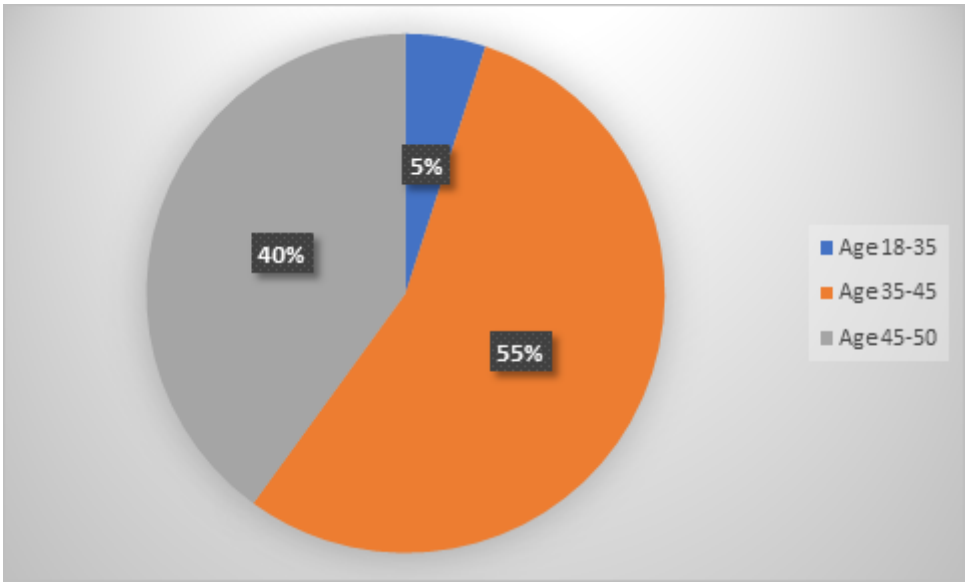

Figure 19

graphical representation of age distribution of subject for collection of GCF and serum.