Original

Evaluation of the Impact of the Clinical Periodontal Status on Volumetric Features of Gingival Crevicular Fluid by using Periotron® 8000

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Abstract: Clinical periodontal status is among the major determinants of the volumetric features of GCF. Previous studies have shown that probing depth, presence/severity of gingival and periodontal inflammation affect the biodynamics of GCF. The aim of the present study was to evaluate the impact of the clinical periodontal status on volumetric features of gingival crevicular fluid. Thirty-six patients were equally divided into healthy group, gingivitis group and periodontitis group. Clinical periodontal status was assessed by recording: papillary bleeding index, probing pocket depth and clinical attachment level. Gingival crevicular fluid (GCF) samples were obtained from 4 sites in each patient. The mean volume of GCF was determined in each group for comparison. Further in periodontitis group, GCF samples were also collected from healthy sites, gingivitis sites and periodontitis sites. Mean GCF volume recorded by using Periotron® 8000 in healthy gingival group, gingivitis group and periodontitis group were: 0.04, 0.09, 0.78 µl respectively. There was increase in GCF volume in a diseased related pattern, healthy gingival group < gingivitis group < periodontitis group. In periodontitis group, the sites with healthy gingiva showed significantly higher GCF volume than healthy gingival group (0.11µl Vs 0.04µl), similarly, the sites with gingivitis in periodontitis group also showed significantly higher GCF volume than gingivitis group (0.32µl Vs 0.11µl). The present study suggests that the volumetric analysis of GCF could be sensitive and reliable indicator of periodontal health. The ability of GCF volume as a chair side measure to differentiate healthy sites from sites with mild disease within the same mouth is considered noteworthy.

Key words: Gingival crevicular fluid, Periodontitis, Periotron 8000

Introduction

Early studies of the association between the GCF volume and health of gingiva were reported by Brill & Loe & Holm-Pederson and they classified sites into categories such as clinically healthy or inflamed and observed an increased GCF volume at these sites designated as inflamed. Later studies graded the level of inflammation and quantified the volume of GCF collected from the site. An association between the two was calculated using linear regression and the results expressed as a correlation coefficient between the clinical assessment of inflammation and the volume of GCF. Since then a considerable part of the interest in GCF seems to be devoted to the unique volumetric features of this biological fluid. In fact, GCF’s specific volumetric features such as the presence of volume and site specific variations in fluid quantity, and the intensity of the factors with the potential to alter volumetric measures make GCF a unique fluid.

Clinical periodontal status is among the major determinants of the volumetric features of GCF. Studies have shown that probing depth (PD), and periodontitis affect the biodynamics of GCF. Therefore, the status of periodontal health and disease can be distinguished by differences in GCF volume. Volumetric measures of GCF are suggested to be sensitive and objective for the monitoring of gingival inflammation and to be reliable indicators of periodontal health. In periodontitis, the potential for GCF measurements to be suitable for assessing pathologic changes seen during pocket formation and in advanced cases as well as in evaluating the effect of periodontal treatment...
is suggested\(^{19}\).

It is clear that periodontal pathology varies from site to site. GCF volumes are suggested to have a disease-related spectrum of volumes\(^{20,21}\). These measures are considered noninvasive, quantitative, objective and site specific means of assessment that can be used for diagnostic purposes\(^{22,23}\). The ability of using GCF volume as a chairside measure to differentiate healthy sites from sites with disease within the same mouth is considered noteworthy\(^{23}\).

When compared to other available methods, electronic volume quantification provides certain clear advantages\(^{24,25}\). The Periotron 8000 (Pro-Flow Inc., Amityville, NY, USA) is the latest version of the device, quantifies the volume of GCF collected on filter papers by measuring the electrical capacitance to interface the machine with a computer for automatic data recording and output, where the inputted data can also be converted to volumes\(^{26,27}\). Thus by adding computer capability, it further improves the reliability of the process of volume quantification\(^{25,26}\). Therefore, the present study was undertaken to evaluate the impact of clinical periodontal status on volumetric features of gingival crevicular fluid by Periotron 8000\(^{8}\).

Materials and Methods

Study population

Forty eight systemically healthy patients (22 males and 26 females) in the age range of 20 to 50 years (mean age, 32.85 years) were selected from the outpatient Department of Periodontics, Sharad Pawar Dental College, Sawangi (Meghe), Wardha. The patients who were smokers or who used any tobacco products, pregnant females or lactating mothers, previous history of antibiotic therapy, anti-inflammatory/ antidepressant drug therapy for the last 6 months prior to the examination were excluded from the study.

Prior to the periodontal examination, the following 3 groups were defined with regards to periodontal status: healthy gingival group- papillary bleeding index score <1 with no obvious changes in color, contour and surface texture of gingiva. Gingivitis group- papillary bleeding index score >1, displaying clinical signs of gingivitis (change in color, contour and surface texture) and sulcus depth d” 3mm. Periodontitis group- mean clinical attachment loss (CAL) e” 5mm) at more than 30% of the sites and/or probing pocket depth (5-7mm). The patients for healthy gingival group were randomly selected from patients who visited our faculty with reasons other than periodontal diseases such as orthodontic purpose, dental caries.

Information concerning dietary status, mouth cleaning habits, systemic background, gingival and periodontal status along with routine clinical details were recorded in a specially designed chart. Patients were examined under good illumination with the help of mouth mirror and William’s graduated periodontal probe.

Prior to initiating study, the purpose and design of the study was explained to patient and informed consent was signed by every patient. The study protocol was first approved by ethical committee of Datta Meghe Institute of Medical Sciences, Sawangi (Meghe), Wardha.

Determination of the clinical periodontal status

Plaque index, gingival index, papillary bleeding index, probing pocket depth (PPD) and clinical attachment level (CAL) were recorded for the entire dentition. All the clinical measurements were made using the manual periodontal probe (William’s periodontal probe). According to the clinical measurements, all the patients were divided into healthy gingival group, gingivitis group and periodontitis group, each group consisting of sixteen patients. To eliminate the risk of interfering with the actual GCF volume, clinical measures were recorded on a separate day before GCF was obtained. All patients were provided with necessary periodontal treatment after collection of data and GCF sample.

To minimize the potential effect of inter-individual volumetric variances, in each patient at least 4 sampling sites were selected. Special care was taken in periodontitis group to ensure that each patient in this group had at least 4 sites of healthy gingiva, 4 sites of gingivitis and 4 sites of periodontitis. Similarly, in gingivitis group- 4 healthy gingival sites and 4 gingivitis sites in each patient.

Collection of gingival crevicular fluid (GCF) samples

Sampling of GCF was done according to the method defined by Rüdin et al\(^{20}\). The site selected for GCF sampling was isolated with cotton rolls to minimize the risk of contamination with saliva. Supragingival plaque was removed to eliminate the risk of plaque contamination. Gentle drying of the site was then performed using a fine bore aspirator tip (Wallace Ltd., Colchester, England) placed on the tooth surface close to the gingival margin. After gentle air drying, GCF was collected using standardized filter paper strips that were inserted 1mm into the sulcus/pocket and left there for 30 seconds. Samples with evidence of gingival bleeding were excluded. To not interfere with the GCF volume, all patients were instructed not to eat anything or brush their teeth for e”1hr before GCF sampling. For standardization, all GCF samples were obtained between 10:00 am and 12:00 pm. GCF sampling was performed at sites with clinically healthy gingiva, gingivitis and periodontitis. According to the site-specific design of the study, the GCF sampling sites in periodontitis group were divided into 3 subgroups: healthy gingival site, gingivitis site and periodontitis site and gingivitis group was also divided into healthy site and gingivitis sites based on the clinical periodontal status.

Determination of GCF volume (Vr)

Before the first reading, an electronic gingival fluid measuring device (Periotron 8000\(^{8}\)) was switched on and was allowed to
warm up before zero adjustment\(^29\). Standardized filter paper strips and a standardized syringe graduated with 10-nl markings were used to generate the calibration curve in triplicate readings with the test fluid (distilled water)\(^26\). To eliminate the potential risk of evaporation, the device was placed in a chairside manner, and paper strips were immediately transferred to the electronic gingival fluid measuring device for electronic volume quantification (within 0 to 2 seconds). The paper strip containing GCF was placed at a standard distance between the electrodes of the device\(^26\). A digital readout (electronic gingival fluid measuring device unit [Pu]) was obtained within 16 seconds and was converted to microliters using specialized software. After each measurement, the electrodes of the device were dried using sterile cotton tissue to eliminate the risk of any contamination between multiple readings. The calibration curve was periodically checked and was generated again based on a 5-Pu change in any particular volume\(^30\). These attempts were considered necessary for precise electronic volume quantification.

**Statistical analysis**

The Levene test was used to analyze the homogeneity of the variances. When variances were not homogenous, the differences among all sites regarding the clinical parameters and Vr were analyzed using the Kruskal-Wallis variance analysis, and subsequent bilateral comparisons were made by Mann-Whitney \(U\) test. Correlations between the mean clinical parameters of a distinct sampling site and the corresponding Vr values were analyzed by simple correlation analysis (Pearson correlation coefficient) and Spearman correlation coefficient.
Forty eight systemically healthy patients (26 females and 22 males) in the age range of 20 to 50 years (mean age, 32.85 years) were selected from the outpatient Department of Periodontics, Sharad Pawar Dental College, Sawangi (Meghe), Wardha for estimation of GCF volume by using Periotron 8000®. According to the clinical periodontal status, all the patients were divided into healthy gingival group, gingivitis group and periodontitis group, each group consisting of 16 patients. Sites in periodontitis group were divided into 3 subgroups: healthy gingival site, gingivitis site and periodontitis site, similarly, sites in gingivitis group were also divided into healthy gingival site and gingivitis site. To minimize the potential effect of inter-individual volumetric variances, in each patient at least 4 sampling sites were selected. Samples with evidence of gingival bleeding were discarded.

Table 1 shows the clinical parameters and GCF-Vr values were significantly lower in healthy sites compared to diseased sites (healthy< gingivitis< periodontitis; p<0.001). The lowest mean Vr value was observed in healthy gingival group, and the highest mean Vr value was observed in periodontitis group (p<0.001). Constant correlations between clinical parameters and GCF-Vr value in all the three groups (p<0.05).

Table 2 shows the mean PPD, PBI and Vr were significantly higher at healthy sites in periodontitis group than gingivitis and healthy gingival group (p<0.001). No significant correlation was found between clinical attachment loss (CAL) and GCF-Vr value in all the three groups.

Table 3 shows the mean GCF-Vr value was significantly higher in gingivitis sites of periodontitis group (0.14±0.01) than gingivitis sites in gingivitis group (0.13±0.01).

Table 4 shows GCF-Vr presented constant correlations with all the clinical parameters in periodontitis group.
Discussion

Forty-eight systemically healthy patients in the age range of 20 to 50 years were divided into healthy gingival group, gingivitis group and periodontitis group by using clinical periodontal status. In each patients, at least 4 sampling sites were selected for the estimation of GCF by using Periotron 8000®. For site, specific estimation of GCF volume, the sampling sites in periodontitis group were divided into healthy gingival sites, gingivitis sites and periodontitis sites as well gingivitis group was also divided into healthy sites and gingivitis sites. Sampling of GCF was done according to the method defined by Rüdin et al.20.

The measurement of early inflammatory changes in the periodontal tissues has lack reliability because of the inherent subjectivity of the various clinical indices used. In an effort to develop a measure of inflammation that is less dependent on subjective estimation than the clinical indices, Brill suggested a procedure for measuring the amount of GCF fluid21. Gingival crevicular fluid (GCF) is an inflammatory exudate that results from the interaction between the host and dental biofilms22. The GCF volume, the net result of gingival and periodontal inflammatory exudates, has been used to evaluate the inflammatory status of the gingival and periodontal tissues in health and disease23,24. The amount of fluid released into the gingival crevice was found to be associated with the amount of inflammation present. Recent studies demonstrated that GCF volume increases with dental plaque accumulation25,26, and decreases after microbial challenge is reduced or removed27.

In the present study, we observed significantly greater GCF volumes in gingivitis group compared to healthy gingival group. Similarly, in periodontitis group, there was significant increase in GCF volume as compared to gingivitis and periodontally healthy groups. In addition, mean GCF volume exhibited significant correlation with all of the clinical parameters in healthy gingival group, gingivitis group as well as in periodontitis group. Previous studies also reported increased in GCF volume both with the severity of gingival inflammation and periodontitis15,19-21. Jepsen et al28, Wright et al29 and Hatipoglu et al30 reported increased in GCF volume in experimental gingivitis model and suggested that this increased GCF volume reflect the level of gingival inflammation. Uttio31 reported that in periodontitis, increased in GCF volume was associated with increased in probing pocket depth and deeper pockets were associated with higher GCF volume. Finding in the present study supports the suggestion that GCF volume increases with gingival inflammation and periodontitis16-19,21,30. In the present study, when GCF volume was compared at healthy gingival sites in all 3 groups, there was significant increase in GCF volume in both gingivitis and periodontitis group compared to healthy gingival group. The discrepancy of GCF volume among clinically healthy sites may possibly arise from variances in the sampling site28, degree of

It is clear that periodontal pathology varies from site to site31. The volume of GCF is suggested to have a disease related spectrum of value16,20. These measures are also considered non-invasive, quantitative & site specific means of assessment that can be used for diagnostic purposes15,16. Therefore, the present study GCF volume at healthy sites, gingivitis sites and periodontitis sites in the same mouth (periodontitis group) were compared. All of the clinical parameters and GCF volume values were significantly lower in healthy sites compared to gingivitis and periodontitis sites (healthy< gingivitis< periodontitis). The lowest mean Vr value was observed in healthy sites (0.8µl) and the highest value was observed in sites with periodontitis (1.42µl). Such site specific variances in Vr are suggested to reflect the level of gingival inflammation31. Lamster32 reported increased in GCF volume with increased in pocket depth. Goodson33 reported higher GCF volume with deep pockets. Booth et al16 found increased in GCF volume in patients with early to advanced periodontitis (1.33µl) compared to healthy (0.33µl). Champagne et al34 suggested that site specific variances in GCF volume could be due to the active or passive state of sites.

The status of periodontal health and disease can be distinguished by differences in GCF volume. Volumetric measures are suggested to be sensitive and reliable indicators of periodontal health17,18. The ability of using GCF volume as a chair side measure to differentiate healthy sites from sites with disease within the same mouth is considered noteworthy17. In periodontitis, the potential for GCF measurements to be suitable for assessing pathologic changes seen during pocket formation and in advanced cases as well as in evaluating the effect of periodontal treatment is suggested19. However, standardization of the distinct location for harvesting GCF from as many available sites as possible, seems to be needed in the interpretation of the clinical diagnostic potential of this biologic fluid. From the analysis of the results, following conclusions were drawn: All the clinical parameters and mean GCF-Vr values were significantly lower in healthy gingival group compared to gingivitis and periodontitis group (healthy< gingivitis< periodontitis; p< 0.001). In periodontitis group, the lowest mean Vr value was observed at healthy gingival sites (0.8µl) and the highest value was observed at sites with periodontitis (1.42µl). Mean PPD, PBI and Vr values were significantly higher at healthy sites in periodontitis group than healthy sites in gingivitis group as well as healthy gingival group (p< 0.001). Mean GCF-Vr values were lower at gingivitis sites in gingivitis group compared with gingivitis sites in periodontitis group.

Conflict of Interest

The authors have declared that no COI exists.
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