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*Original Research Article*

# Comparative Evaluation of Salivary Flow Rate, Ph, Buffering Capacity, Total Protein and Albumin Levels in Chronic Periodontitis Patients: A Clinico-Biochemical Study

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## ABSTRACT

**Aim and objectives**: This study was designed to compare the total protein concentration, albumin, flow rate, pH, buffering capacity of saliva in healthy subjects, patients with gingivitis and chronic periodontitis.
**Materials and methods**: A total of 60 patients were selected and were divided into three groups (20 subjects each) as controls, gingivitis and chronic periodontitis. Plaque index, gingival index, modified sulcular bleeding index, probing pocket depth and clinical attachment level were recorded. The unstimulated whole saliva was collected from patients and flow rate was noted down during collection of the sample. Estimation of total salivary protein and albumin was performed by colorimetric method. The pH estimation was done using pH meter and buffering capacity was analysed with the titration method.
**Results and Conclusion**: The results of this study showed significant rise in salivary total protein and albumin concentration in gingivitis and chronic periodontitis patients whereas pH, buffer capacity and flow rate did not show any significant changes. Thus, salivary total protein and albumin concentration may serve as an important biochemical parameter of inflammation of the periodontium.

***Keywords-*** Saliva, salivary protein, gingivitis, periodontitis.

## INTRODUCTION

Saliva influences oral health both through its non-specific physico-chemical properties, as well as through more specific effects. [1] Human salivary proteins have a wide range of functional properties including the immune response, inhibition of calcium precipitation, taste perception, digestion, cell proliferation, signal transduction, chemotaxis and cell motility. [2] Therefore, salivary total normal protein concentration is vital for good oral health and a sustained change in it for any reason adversely affects the oral health of these patients.

Gingivitis is a disease that is characterized by inflammation of the gingival tissue, which can progress to periodontitis and tooth loss. [3] Biochemical contents like various proteins, especially albumin considered as a serum ultra filtrate to the mouth is of significance because,

salivary albumin and therefore salivary proteins have been shown to be increased in medically compromised patients and in local chronic inflammatory conditions like periodontitis where salivary protein and albumin concentration changes serve as indicators of plasma protein leakage and consequently affect oral health status. [4]

Saliva as a mirror of oral and systemic health is a valuable source for clinically relevant information because it contains biomarkers specific for the unique physiological aspects of periodontal / peri-implant disease, and qualitative changes in the composition of these biomarkers could have diagnostic value by identifying patients with enhanced disease susceptibility, identifying sites with active disease, predicting sites that will have active disease in the future and / or serving as surrogate end points for monitoring the effectiveness of therapy. [5] Saliva, contains locally-derived and systemically- derived markers of periodontal disease, and at the same time it can be easily collected, non-invasive, readily available and does not require specialized equipment or personnel. Thus, offering the basis for a specific diagnostic test for periodontitis. [6] Hence, the aim of the present study was to analyze the salivary total protein, albumin, pH, buffering capacity and flow rate in both normal subjects and in patients with gingivitis and periodontitis using simple biochemical methods.

## MATERIALS AND METHODS

This study was conducted in the Department of Periodontology, Rungta college of Dental sciences and research, Bhilai, Chhattisgarh. A total number of 60 subjects were included in the study and were divided into 3 groups: 20 subjects with gingivitis, 20 with chronic periodontitis and 20 healthy subjects. The groups were divided based on the condition of the periodontal tissues which was evaluated by clinical as well as by radiographic examination. A complete periodontal examination included assessment of bleeding index, gingival index, probing pocket depth, clinical attachment level and panoramic radiographs.

### Inclusion criteria

Subjects with minimum complement of 20 natural teeth were included in the study .In control group, patients with GI score $\leq 1$ with no loss of attachment were included.
In the gingivitis group, subjects with bleeding on probing and GI score $\geq 1$ in more than 30% of sites with no loss of attachment were included.
Subjects having clinical attachment loss of 4mm with radiographic evidence of bone loss in more than 30% of the sites were included in the chronic periodontitis group.

### Exclusion criteria

1. Subjects with history of any systemic diseases or conditions.
2. Subjects with history of any salivary glands diseases.
3. Subjects with any oral infections (i.e. herpes or candida).
4. Intake of antibiotics or anti-inflammatory drugs within 6months prior to the study.
5. Pregnant or lactating women.
6. Subjects with history of smoking or any form of tobacco and alcohol consumption.
7. Individuals who had received any periodontal treatment in the past six months prior to the study were also excluded from the study.

### Collection of saliva

Subjects were instructed not to brush their teeth, or eat or drink one hour before the time of saliva collection. Unstimulated whole saliva was collected by spitting method between 11am and 12 noon to avoid diurnal variation. Subjects were seated in an upright position, with instructions to accumulate the saliva in the floor of the mouth and to spit without stimulation into the sterile container. The collected samples were immediately taken for biochemical analysis. Approximately, 5ml of saliva was

collected from each patient. Flow rate of saliva was calculated as volume collected divided by the time required for the collection.

**Estimation of pH and buffering capacity:**

pH of saliva was measured by using manual pH meter with the help of pH meter .buffering capacity of saliva is measured by titration method in which 0.5 ml of saliva was added to 1.5 ml of 5mmol/ l HCl. The mixture was vigorously shaken and centrifuged for one minute and then it was allowed to stand for 10 minutes. Then the final pH was measured by using manual pH meter.

**Salivary protein estimation**:

Salivary protein estimation was done based on Biuret method. Protein forms a coloured complex with cupric ions in alkaline medium. Based on this principle salivary protein estimation was done by mixing undiluted saliva with the reagent provided and measuring the coloured product using a photoelectric colorimeter at a wavelength of 546 nm.

**Salivary albumin estimation**: This was done by Bromocresol green method in which the reaction between albumin in saliva and the dye Bromocresol-green produces a change in colour that is proportional to the albumin concentration. It was estimated using a photoelectric colorimeter at wavelength of 620 nm.

**Statistical analysis**

The comparison between the groups was carried out by Fisher's test (ANOVA).

## RESULTS

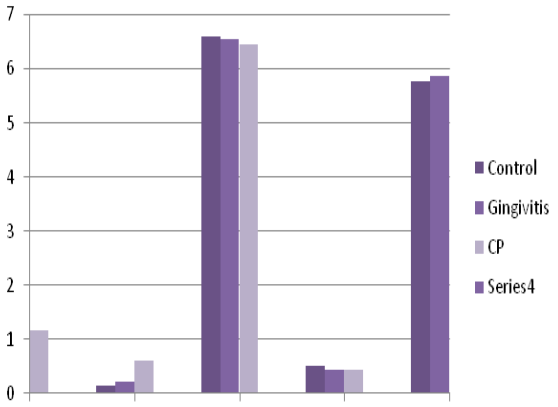
Table 1 and graph 1 estimate the significance of different parameters in the three groups using Fisher's test.

Salivary total protein and albumin estimation were shown to be very highly significant. Salivary total protein in the controls, gingivitis and periodontitis group was 0.72 g/mL (SD=0.23), 1.05 mg/ml (SD=0.19) and 1.16 g/mL (SD=0.23) respectively. Total mean salivary albumin for controls, gingivitis and periodontitis patients was 0.14 (SD=0.05), 0.22 (SD=0.11) and 0.60(SD=0.21) mg/ml respectively. pH and buffer capacity did not show any significant changes among the control, gingivitis and periodontitis groups.

**Table 1: Estimating the significance of the parameters in the study**

| Variables | Mean | SD | F value | P value |
|---|---|---|---|---|
| **pH** | | | | |
| Control | 6.62 | 0.21 | 1.072 | 0.32 * |
| Gingivitis | 6.57 | 0.22 | | |
| Periodontitis | 6.455 | 0.25 | | |
| **Buffer Capacity** | | | | |
| Control | 5.781 | 0.12 | 1.85 | 0.25 * |
| Gingivitis | 5.869 | 0.19 | | |
| Periodontitis | 5.798 | 0.14 | | |
| **Total Protein** | | | | |
| Control | 0.72 | 0.13 | 21.64 | 0.0001 † |
| Gingivitis | 1.05 | 0.19 | | |
| Periodontitis | 1.165 | 0.23 | | |
| **Albumin** | | | | |
| Control | 0.1435 | 0.05 | 62.05 | 0.0001 † |
| Gingivitis | 0.223 | 0.11 | | |
| Periodontitis | 0.602 | 0.21 | | |
| **Flow Rate** | | | | |
| Control | 0.50 | 0.21 | 1.21 | 0.27* |
| Gingivitis | 0.45 | 0.22 | | |
| Periodontitis | 0.44 | 0.25 | | |

* - Nothing significant
† - Highly significant




**Graph 1. Comparison of variables between three groups namely: control, gingivitis and chronic periodontitis (CP).**

## DISCUSSION

The present study was carried out to estimate the salivary total protein, albumin, pH, buffering capacity and flow rate in both normal subjects and in patients with gingivitis and periodontitis using simple biochemical methods.

Total protein is a vital component of saliva and is responsible for most of its functions like lubrication, physical protection, cleansing, buffering, maintenance of tooth integrity, taste and

digestion and antibacterial activity. [7] The major factors affecting the protein concentration and composition of whole saliva are the salivary flow rate, protein contributions of the glandular saliva and crevicular fluid proteins.

According to the results of our study, there was a rise in total salivary protein concentration in the gingivitis and periodontitis group and the mean values in the controls, gingivitis and periodontitis were 0.72 mg/mL(SD=0.13), 1.05 mg/mL (SD=0.19) and 1.16 mg/mL(SD=0.23) respectively.

Another study was conducted by Hensken et al. [8] to evaluate the salivary protein, albumin and cystatin concentrations in subjects with healthy periodontium and subjects with gingivitis or periodontitis showed similar studies with the mean total protein values in the controls, gingivitis and periodontitis subgroup as 1.06 mg/mL (SD=0.25), 1.49 mg/mL (SD=0.58) and 2.21 mg/mL (SD=1.0) respectively. Both the periodontitis and gingivitis subgroups showed 1.8 and 1.3 times rise in the total protein value respectively, when compared with that of the controls. The increased levels could partly be also due to an increased leakage of plasma proteins into saliva due to inflammation as suggested in his study.

Another study by Hensken et al. [9] showed that levels of total protein and cystatin activity as well as the levels of glandular derived proteins amylase and cystatin C were significantly higher in whole and parotid saliva of subjects with periodontitis than in healthy controls. The increased protein levels in the test groups could be due to the inflammatory process that activates the sympathetic system to enhance the synthesis and secretion of some proteins (as evidenced by increased amylase levels) thereby increasing the protective potential of saliva against the diseases. Thus, the elevated protein levels are most likely due to enhanced synthesis and secretion by the individual glandular saliva. Also, glandular-derived proteins, Cystatin C and amylase showed significant rise in periodontitis subjects, proving the glandular origin of these proteins.

Our results are also in agreement with Shaila et al. [4] who found increase total protein levels in gingivitis and periodontitis patients as compared to control group. In this study, the mean values in the controls, gingivitis and periodontitis subgroup was 0.86 g/mL (SD=0.21), 1.19 g/ml (SD=0.23) and 1.59 g/mL (SD=0.48) respectively. The explanation given in their study is that periodontal microbes trigger inflammatory response which results in higher levels of salivary albumin and total protein. Therefore, estimation of total protein concentration using the Biuret method is a valuable biomarker for the diagnosis of periodontal disease.

In our study a significant increase was seen in salivary albumin levels with the mean values of 0.14 mg/ml (SD=0.05), 0.22 mg/ml (SD=0.11) and 0.6 mg/ml. (SD=0.21) in the controls, gingivitis and periodontitis group respectively. Salivary albumin is described as a component of the acquired pellicle and it is found in a complexed form with proline rich glycoproteins of pellicle which play an effective role in lubrication of oral tissue surfaces. There is much evidence to substantiate the fact that increased albumin concentrations during inflammation and periodontal breakdown are found in saliva and as reported in studies of Hensken et al. [8] with mean values in the controls, gingivitis and periodontitis subgroups as 0.08 mg/mL (SD=0.05), 0.30 mg/mL (SD=0.30) and 0.67 mg/mL (SD=0.50) respectively.

Also in study by Shaila et al. [4] total mean salivary albumin for controls, gingivitis and periodontitis patients was 0.09 (SD=0.04), 0.24 (SD=0.09) and 0.44 (SD=0.12) mg/mL respectively showing increased level in patients of gingivitis and periodontitis.

Altered periodontal health also plays a role in salivary flow rate. The salivary flow is directly related to the salivary

consistency. Thus, greater the salivary flow, greater the consistency and greater the cleaning and diluting capacities; therefore, if changes in health cause a reduction in salivary flow, there would be a drastic alteration in the level of oral cleaning. [10] The findings of present study however did not show any differences in the flow rate in comparison between the groups.

The normal salivary pH is from 6 to 7, and varies in accordance with the salivary flow from 5.3 to 7.8.There are various sources of hydrogen ions in oral fluids: secretion by the salivary glands in the form of organic and inorganic acids, production by the oral microbiota or acquisition through foods. These ions influence the equilibrium of calcium phosphates in the enamel. The higher the concentration of hydrogen ions, the lower the pH, and vice versa. [10] In present study, in terms of pH and buffering capacity no significant difference was seen between the groups.

Thus, it can be said that the findings of the present study suggest that salivary protein and albumin may serve as a marker of inflammation of the periodontium. However, there are some limitations of the study including the short sample size of the study and lack of interventional studies. Further long term clinical trials would be required to substantiate the findings obtained in this study.

## CONCLUSION

The study showed increased levels of salivary albumin and total protein in gingivitis patients and chronic periodontitis patients by using simple biochemical tests. The results provide evidence that salivary glands may respond to periodontal diseases by enhanced synthesis of some acinar proteins, thereby increasing the protective potential of saliva. Thus, salivary albumin and total protein may serve as an important biochemical parameter of inflammation of the periodontium.

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