Salivary total protein and amylase levels in patients with gingivitis and chronic periodontitis

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Abstract
Inflammation of gums and its supporting structure is known as periodontitis. As this is strictly related to oral health, it will ultimately be useful to study oral secretion with some of its biochemical parameters, which are usually studied in serum. The usefulness of this parameters to gauge oral health especially with reference to periodontitis was determined in present study. The study was conducted in Mumbai based dental college. The aim of the study was to evaluate the changes in the levels of total proteins and salivary amylase with increase in dental carries and to compare the same with healthy subjects. Students ‘t’ test was applied. The study comprised of total 90 subjects, out of which 30 had gingivitis, 30 had periodontitis and 30 normal healthy individuals respectively. Both were age and sex matched. The result showed response of salivary glands to periodontal disease by enhancing synthesis of some protein, thereby increasing the protective potential of saliva.

Keywords: periodontitis, chronic periodontitis, gingivitis, salivary total protein, salivary amylase

Introduction
In recent years people have become more conscious about oral health. Periodontitis (gum disease/ pyorrhoea), which is very common among people, is a set of inflammatory diseases affecting the tissues surrounding the teeth. Gingivitis is a non-destructive, reversible inflammatory condition whereas chronic periodontitis is irreversible condition leading to destruction of connective tissues and alveolar bone. These occur as a result of the interaction between a pathogenic bacterial biofilm and host derived inflammatory cells and molecules [1]. Therefore, diverse researches are being performed to evaluate the severity of the disease as well as to pinpoint the risk of the disease [2].

Saliva is a body fluid, secreted by three pairs of major salivary glands (parotid, submandibular and sublingual) and by many of minor salivary glands. Saliva contributes to digestion of food and to the maintenance of oral hygiene. Absence of normal salivary function leads to dental caries, gum disease (gingivitis and periodontitis) and other oral problems [3]. Various microorganisms are inhibited by salivary amylase [4]. Alteration in protein composition of whole saliva is also observed in patients with periodontal disease [5].

There is very scanty literature available regarding the relation between total protein and amylase. Therefore, this study was designed to assess and compare the levels of salivary total protein and amylase in healthy, gingivitis and chronic periodontitis subjects.

Materials and Methods
The study compromised of total 90 subjects, out of which 30 had gingivitis, 30 had periodontitis and 30 normal healthy individuals respectively. Both age and sex matched. The groups were divided based on the clinical and radiographic examination of the periodontal tissues.

Inclusion and exclusion criteria
Subjects with gingivitis and periodontitis without any systemic disease were included. Subjects with systemic disease like diabetes mellitus, HIV, etc. were excluded from the study. Subjects who consumed tobacco in any forms (smoking, chewing) and who had mumps, oral tumours etc. were also excluded from the study. The study was approved by the Institutional ethics committee. Written informed consents were obtained from the subjects.

Saliva Collection
The subjects were given prior information regarding collection of saliva.
Unstimulated saliva was collected from 10-11 am. Subjects were asked to refrain from eating and drinking anything two hours prior collection. The patients were instructed to allow the saliva accumulate in the floor of the mouth and to spit without stimulation into sterile container. The samples were stored at 4°C. The collected samples were immediately taken for biochemical analysis \[1\].

Total protein was evaluated by Folin Lowry method \[6\] and amylase was estimated by DNSA (3, 5-dinitrosalicylic acid) method \[7\].

**Statistical analysis**
Statistical analysis was done using students ‘t’ test. The values obtained were tabulated and mean and standard deviation (SD) of both the parameters was calculated. The test of significance applied was ANOVA (Analysis of Variance) test to compare the levels of total protein and amylase in saliva of patients and healthy subjects. p value ≤ 0.001 was considered as statistically significant.

**Result**
The study exhibited remarkable higher levels of total protein in gingivitis and periodontitis patients when compared to healthy subjects (Graph 1, Table 1). However, the levels were much higher in periodontitis group compared to gingivitis group. A phenomenal increase in levels of salivary amylase was also observed in gingivitis and chronic periodontitis group compared to control group (Graph 2, Table 2). Higher levels were noted in periodontitis group compared to gingivitis group.

**Discussion**
Salivary amylase, an enzyme which belongs to class of hydrolases, catalyses the hydrolysis of α-1-4 glycosidic bonding of polysaccharides \[4, 8\]. Amylase, a calcium containing enzyme, facilitates dietary starch hydrolysis to provide maltose and thereby glucose. This provides supply of glucose for metabolism of plaque microorganism in close proximity to the tooth surface resulting in lactic acid production. This ultimately leads to lowering pH and causing oral health issues \[8\].

Our study is in accordance with other studies which showed an increase in amylase levels in patients with gingivitis and periodontitis \[4, 9\]. The amylase levels in the gingivitis group were also seen to be high (251.67U/ml, SD ± 52.54), whereas in periodontitis group showed significant elevation (427.22U/ml, SD ±59.78) (graph 2) as compared to healthy control.

The increased levels may be due to the response of salivary glands to inflammatory diseases like gingivitis and periodontitis resulting in increased synthesis and secretion of certain acinar proteins like α-amylase so as to enhance the oral defence mechanism \[9, 10\].

α-amylase is a major lipo-polysaccharide binding protein of aggregatibacter actinomycetes comitants and porphyromonas gingivalis and interferes with bacterial adherence and biofilm formation. Therefore, higher concentration of salivary amylase in our study suggests to be an important defence molecule essential for innate immunity in oral cavity \[11\].

The results of our study showed significant increased levels of total protein in gingivitis group (273.35%, SD 57.94), whereas the periodontitis group also showed remarkable elevation (469.47%, SD 62.12) (graph 1) as compared to
The increase levels may be due to the inflammatory process that activates the sympathetic system to enhance the synthesis and secretion of some proteins thereby increasing the protective potential of saliva against the disease. The increased levels could also be partly due to an increased leakage of plasma proteins into saliva due to inflammation [9, 12].

In our study, a significant positive correlation was also observed among proteins and amylase indicating that they are implicated in host response. This is in accordance with other studies which also showed a positive correlation among proteins, mucin and amylase [4, 9].

Conclusion
The study showed increased levels of salivary total protein and amylase in gingivitis and chronic periodontitis subjects when compared to healthy subjects. However, the levels were significantly much higher in periodontitis subjects compared to gingivitis.

The results provide evidence that the salivary glands may respond to periodontal disease by enhancing synthesis of some proteins, thereby increasing the protective potential of saliva. Detecting certain protein biomarkers in saliva can help to determine if the subjects are at future risk of periodontitis and proper oral care and therapy can be provided to prevent its progression to periodontitis. Thus, it can be hypothesized that various enzyme inhibitors might be therefore useful as a part of host modulation therapy in the treatment of periodontal diseases.

References