Alterations in Whole Saliva Constituents in Patients with Diabetes Mellitus and Periodontal Disease

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ABSTRACT

Background and objectives: Neglected by dentists and ignored by physicians, saliva is the least appreciated of all body fluids. Yet, this secretion plays a vital role in the integrity of the oral tissues in the selection, ingestion and preparation of food for digestion and besides a whole lot of functions, in one’s ability to communicate with one another. Saliva has proven to be a discriminating element in forensic sciences, an effective indicator of acute diseases of salivary glands and also a promising probe for drug monitoring. Because of multiplicity of functions served by saliva along with various physiologic processes involved, salivary secretions have long enjoyed a modest popularity as a research field. With an increase in investigator interest, it is becoming apparent that many systemic diseases affect salivary gland function and/or composition potentiating its probable role as an indicator of systemic disease.

Materials and methods: This study has been undertaken to assess the possible variations in the salivary components in patients with diabetes mellitus and periodontal disease and to ascertain their role in the progression and severity of the disease process. Ninty patients from the OPD were selected for the study after taking an informed consent. These were divided into three groups. Group I consisted of patients with diabetes mellitus and periodontal disease, group II consisted of patients with periodontal disease alone, and, group III the control group. Around 10 ml unstimulated saliva obtained from these subjects was analyzed for salivary sodium, potassium, alpha-amylase, albumin, total proteins and IgA levels. The results were analyzed using the student’s t-test.

Results: All the salivary components assessed were markedly increased in group I than group II which showed slightly higher values than group III.

Conclusion: Diabetes mellitus acts as a modifying and aggravating factor along with presence of local etiological factors to increase the severity of periodontal disease. Alterations in salivary composition are seen as a result of changes in the oral mucosa due to diabetes mellitus which in turn affect the severity of periodontal disease.

Keywords: Diabetes mellitus, Periodontal disease, Salivary composition.

INTRODUCTION

The oral cavity holds great many secrets which are reflected purely in its appearance and function. Hence, very aptly the oral cavity is said to be the mirror of systemic diseases as many a systemic diseases can be identified on the basis of their oral manifestations alone.

Diabetes mellitus is one such complex multifactorial genetic disorder of unknown etiology characterized by increased insulin secretion and decreased salivation. In addition to these the most common oral manifestation is the presence of periodontal disease, whose presence and severity can often lead the oral physician to suspect an underlying diabetes mellitus.

This association of diabetes with periodontitis is attributed to the alterations in the permeability of the basement membrane, improper neutrophil chemotaxis, collagen synthesis, genetic predisposition and an increased susceptibility to periodontal pathogens.

Salivary glands are important glands of the digestive system and serve functions which influence oral health in general. The salivary secretions are also very convenient for extirpation and collection. With an increase in investigator interest it is becoming apparent that many systemic diseases affect salivary gland function and/or composition potentiating its probable role as an indicator of the disease process. Also with the advent in sensitive immunochemical assays continuing, the compositional profile of human salivary secretions is expanding considerably, thus, establishing a range of normal values for a variety of intrinsic and extrinsic salivary components representing a stepping stone to use saliva as a diagnostic tool to assess oral health status.

AIMS AND OBJECTIVES

1. To assess the possible alterations in salivary composition in patients with diabetes mellitus.
2. To assess presence and severity of periodontal disease in patients with diabetes mellitus.
3. To assess the possible role of saliva and its composition in initiation and progression of periodontal disease in diabetes mellitus.
MATERIALS AND METHODS

This study was conducted in the Department of Oral Medicine and Radiology, KLES’s Institute of Dental Sciences, Belgaum. A total of 90 patients were selected from the outpatient department after an informed consent was obtained from them.

Clinical Evaluation

Inclusion Criteria

Group IA: Patients with noninsulin-dependent diabetes mellitus (NIDDM)

IB: Patients with insulin-dependent diabetes mellitus (IDDM)

Group II: Patients with periodontal disease and no underlying systemic disease (Figs 1 and 2)

Group III: Control

Exclusion Criteria

• Patients with any other underlying systemic disease with or without diabetes mellitus

• Patients undergoing any periodontal therapy or antibiotic therapy 6 months prior to the study.

All the patients participating in the study were screened for diabetes mellitus by determining the fasting and postprandial blood glucose levels. Their periodontal status was ascertained by measuring the probing depths and grading the periodontal disease using the Loe and Sillness gingival index.

SALIVARY SAMPLING PROCEDURES

1. Collection of saliva was done in the morning prior to the patients consuming their breakfast. They were asked to accumulate saliva in their mouth for 5 to 7 minutes following which the entire resting unstimulated saliva was expectorated into two measuring cylinders of 5 ml each.

2. Biochemical assay

a. Quantization of salivary sodium and salivary potassium was done using flame photometry. Standard solution of sodium (140 meq/lt) was prepared by dissolving 58.5 mg NaCl in 1 liter of glass-distilled water. Standard solution of potassium (7 meq/lt) was prepared by dissolving 7.4 gm of KCl in 1 liter of distilled water.

b. Quantization of salivary amylase was done using photoelectric colorimetry. As the amylase is activated by chloride ions, the dilutions were made in physiologic saline. The alpha-amylase was allowed to react under-defined conditions. A blue coloration after addition of iodide solution was compared with the control to give a measure of the alpha-amylase activity.

c. Quantization of salivary total proteins was done using the biuret method. The salivary sample was mixed in the biuret working reagent and the readings were calculated using a green filter at 546 nm with a photoelectric colorimeter. Proteins reacted with the biuret reagent to form a violet colored complex which was directly proportional to the concentration of proteins in the saliva.

d. Quantization of salivary albumin was done using the bromocresol green dye method. Binding of the albumin with the dye in a citrate buffer resulted in a green colored complex which was proportional to the albumin concentration in the saliva.

3. Radioimmunoassay

The single radial immunodiffusion (SRID) technique first described by Mancini in 1965 was used for the quantization of salivary IgA levels. In this technique the protein antigen diffuses radially from a point application into an antibody containing gel. A circular precipitate is formed at the point to equivalence the area encompassed by the precipitation is proportionate to the concentration of the antigen. IgA immunoglobulin was quantified using single radial immunodiffusion assay. In this a circular well of the protein antigen formed around the antibody concentration. The diameter($d^2$) was plotted against the antigen concentration and a straight line was obtained using the equation:

$$d^2 = k \cdot (C_{ag}) + S_0$$

the intercept ‘$S_0$’ is a function of the antigen well diameter and the volume of antigen applied.
RESULTS

Gingival index measured in group I (1.99 ± 0.26) was the highest followed by the scores of group II (1.74 ± 0.08) and group III (0.77 ± 0.06). These findings were in corroboration with studies conducted by Belting et al,7 Yavuzyilmaz et al9 Erhan Firtal1i9 and O’leary et al.10 Our findings clearly indicated that the presence of an underlying systemic disease like diabetes mellitus may lead to initiation or rapid progression of periodontal disease.

1. Salivary sodium concentration of group I (20.13 ± 9.99) was comparable to the values obtained in group II (17.9 ± 8.80) and group III (12.7 ± 6.61) which confirmed our hypothesis that the increased concentration of salivary sodium was a result of local etiology and that the diabetes mellitus added to its severity.

2. Salivary potassium concentrations in group I (19.5 ± 8.49) was higher than group II (12.09 ± 5.55). It was surprising to note that the values obtained in group III (14.25 ± 5.17) were slightly higher than group II. Sharon et al11 in their study found an increase in salivary potassium in their diabetic group than in the nondiabetic group. They suggested that since diabetes mellitus is associated with autonomic neuropathy, sympathetic-parasympathetic imbalance may exert a continuous stimulation of the salivary glands, thus the increased salivary potassium levels. Also adrenaline, an essential factor in the recovery mechanism from hypoglycemia played a key role in creating acinic cell membrane stimulation and causes high levels of salivary potassium.

3. Salivary total proteins in group I (0.64 ± 0.53) were increased in comparison to group II (0.39 ± 0.22) and group III (0.37 ± 0.18). This increase in the diabetic group was attributed to the basement membranopathy in the salivary glands than due to periodontal disease. Lack of synthesis of a specific proteoglycan, heparin sulfate leaves a newly formed basement membrane functionally defective. This heparin sulfate serves as a negatively charged shield which prevents serum anions from permeating the membrane. Thus, the aberrant diabetic membrane lacking this shield becomes more permeable and cationic. Similar views have been put forth by Mandel,12 where he proposed that the basement membrane presented with increased passage of proteins from the salivary glands into their secretions and this increased permeability leads to an enhanced leakage of serum derived components into the saliva via the gingival crevice.

4. Salivary albumin levels in group I (0.33 ± 0.30) was higher than group II (0.2 ± 0.11) and group III (0.21 ± 0.09).

5. Salivary amylase levels in group I (631.31 ± 337.61) were significantly higher than in group II (340.23 ± 300.54) and group III (232.66 ± 84.54). This increase in the alpha-amylase in group I was attributed to the fact that the end product of the alpha-amylase hydroxylation resulted in the formation of glucose and other monosaccharide. Increased glucose secretion has been attributed to glandular secretory changes and increased glucose secretion in the crevicular fluid which eventually alters the plaque environment leading to the growth of specific microorganisms.12 In the study by Yavuzyilmaz et al9 contrary results were obtained which they explained due to hormonal and metabolic changes in diabetes which can significantly affect salivary gland composition and function.

6. Secretory IgA levels in group I (12.45 ± 4.78) were higher than the values in group II (9.10 ± 4.55) and group III (2.98 ± 0.76). Thus, it was evident that diabetes mellitus an immunodeficient disease quantitatively and qualitatively increased the microflora which resulted in an exaggerated response of IgA. This in turn increased the accumulation of plaque and calculus resulting in pronounced periodontal inflammation. Our study was in accordance with studies conducted by Sharon et al,11 Yavuzyilmaz et al,5 Marder et al13 and Lindstrom et al.14

7. Probing depth in group I (5.2 ± 0.31) was significantly higher than group II (4.28 ± 6.35) and group III (2.21 ± 0.19). These findings were consistent with the findings of Marder et al,13 Aryeh BH15 and Yavuzyilmaz et al.8 Our findings confirmed the general hypothesis that the degree of periodontal inflammation was more severe in the diabetic group than group II and group III.

DISCUSSION AND CONCLUSION

With recent times, the potential usefulness of analysis of the salivary secretions in diagnosis and prognosis has been thoroughly explored. Modern methods applied to this secretion may provide information that is different from other body fluids. Earlier studies have been conducted using stimulated salivary samples,13 stimulated whole salivary samples16 and whole unstimulated salivary samples8 in an attempt to determine the possible relationship between the altered salivary secretions and mucosal changes occurring subsequent to diabetes mellitus. According to Finney et al, periodontal disease has been considered the 6th ‘opathy’ associated with diabetes after ‘retinopathy’, ‘nephropathy’, ‘vasculopathy’ and ‘peripheral and autonomic neuropathies’.17

In the current investigation conducted at the Department of Oral Medicine and Radiology, KLES’s institute of Dental Sciences, Belgaum, we estimated the alterations in whole salivary constituents, such as salivary sodium, potassium, total proteins, amylase, albumin and secretory IgA. And a possible explanation was sought to the prevalence and severity of periodontal disease in diabetes mellitus and the role of saliva that brought about these changes. Whole unstimulated saliva was used and analyzed. The results obtained potentiated our claim that there was a definitely positive alteration seen in the salivary composition in diabetic and nondiabetic individuals with or without periodontal disease. Also the severity of periodontal disease as seen in diabetic patients was more accentuated than in the nondiabetic group. The duration of
diabetes and the type of medication did not have any positive correlation with either the severity of the periodontal disease or the composition of whole salivary constituents. A reasonable interpretation of the obtained evidence indicated that diabetes when a complication of periodontitis acts as an aggravating factor in the severity of the periodontal disease.

In conclusion, the ability to use saliva as a diagnostic tool is of great value as the sample can be collected noninvasively, does not require professional personnel. Moreover, saliva can be collected at the point of its origin and manufacture and so is unaffected by collection or storage in the body. Establishing a range of normal values for a variety of intrinsic and extrinsic salivary components will enable investigators and clinicians in a variety of disciplines to use saliva as a diagnostic tool. As this study proves that alterations in salivary composition are noted in a number of clinical situations and systemic diseases and these can be easily determined by the personnel to the desired effect.

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