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Evaluation of Salivary pH and Microbial Load in Periodontitis Patients with Uncontrolled Type I and Type II Diabetes to that of Periodontitis Patients without Diabetes

Dr Mohammed Salman, BDS, (MDS)[1], Savita Sambashivaiah, MDS[2]

[1]Post Graduate, Department of Periodontology, Rajarajeswari Dental College & Hospital, Bangalore

[2]Professor & Head Of The Department, Department of Periodontology, Rajarajeswari Dental College & Hospital, Bangalore

Abstract: ***Aim: To evaluate the level of salivary ph and total bacterial count in periodontitis patients with type I and type II diabetes mellitus to that of periodontitis patients without diabetes. Materials & Methods: The study population consisted of 45 patients randomly allocated into 3 groups, Group I: 15 subjects with chronic periodontitis alone.Group II: 15 subjects with chronic periodontitis and uncontrolled type 1 diabetes.Group III: 15 subjects with chronic periodontitis and uncontrolled type 2 diabetes.Unstimulated saliva sample was collected. The pH of the saliva was determined using pH strip and total bacterial count was evaluated from the collected saliva sample. Statistical analysis was carried out. Results: The results showed that the pH of saliva was more acidic in group II compared to that of group I as well as group III. Assessment of total microbial load in saliva showed statistically significant increase in the micrococci count in group III compared to group I and group II. Conclusion: The results of the present study concluded thatthere was a significant reduction in the salivary pH in uncontrolled type I diabetes patients with periodontitis, compared to that of non-diabetic subjects with periodontitis. Total microbial count of micrococci in uncontrolled type II diabetes patients with periodontitis was more compared to other groups***

Keywords: Diabetes, Periodontitis, Salivary pH, Microorganisms

1. Introduction

Diabetes mellitus is a disease of metabolic dysregulation, primarily of carbohydrate metabolism,characterized by hyperglycemia resulting from defect in insulin secretion, insulin action, or both. The chronic hyperglycemic state of diabetes is associated with long-term damage, dysfunction, and failure of different organs, especially the eyes, kidneys, nerves, heart, and blood vessels.[1] Prolonged hyperglycaemia in diabetes can compromise the immune, cardiovascular, renal and ophthalmic systems, thus producing an array of complications like neuropathy, peripheral vascular disease, renal disease, retinopathy and coronary heart disease.[2]

Type I diabetes mellitus,which accounts for only 5–10% of those with diabetes, previously encompassed by the terms insulin dependent diabetes, type 1 diabetes, or juvenile-onset diabetes, results from a cell-mediated autoimmune destruction of the insulin-producing β cells of the pancreas. WhereasType 2 Diabetes Mellituswhich accounts for 90–95% of those with diabetes, previously referred to as non–insulin dependent diabetes, type 2 diabetes, or adult-onset diabetes, encompasses individuals who have insulin. Most patients with this form of diabetes are obese, and obesity itself causes some degree of insulin resistance.[3]

It has been noted that the salivary glands are also affected directly or indirectly. Reported oral health complications in patients with diabetes, which are usually encountered by practitioners include xerostomia, tooth loss, gingivitis, periodontitis, odontogenic abscesses and soft tissue lesions of the tongue and the oral mucosa.[2] A study done by *C Seethalakshmi* et al.(2016) showed that diabetes mellitus subjects had decreased salivary pH when compared to that of control group which may be attributed to the metabolic changes in diabetes mellitus patients resulting in acidic pH.[4]

A link between diabetes and periodontitis in adults has been confirmed.[5] In fact periodontitis has been considered as the sixth complication of diabetes.[6] It is well-established that diabetes increases the prevalence, severity, and progression of periodontal disease, [7, 8, 9] vice versa, the fact that periodontal disease may complicate the severity of diabetes by worsening the degree of glycemic control has also been proven.[10, 11]

A study by *Takahashi* et al.(1990,1997) on the effect of pH on the growth of microorganisms showed that *P. gingivalis*grows at a pH of 6.5-7.0, *P. intermedia*grows at a pH of 5.0-7.0 and *F. nucleatum*grows at a pH of 5.5-7.0. As diabetes reduces salivary pH it creates a favourable atmosphere for the growth of these bacterias which cause periodontitis.[12, 13]
Numerous oral changes have been described in diabetic patients, including alteration in flora of oral cavity. In uncontrolled diabetes the function of the immune cells like Neutrophil adherence, chemotaxis, and phagocytosis are often impaired, which may inhibit bacterial killing in the periodontal pocket and significantly increase the periodontal pathogens. It has been shown that diabetic patients with severe periodontal disease have more complications of diabetes and less effective metabolic control compared with diabetic patients with healthy gingiva.[14]

Hence this study aims to compare and evaluate the salivary pHbetween chronic periodontitis patients withuncontrolled

type I and type II diabetes to that of patients with chronic periodontitis alone and also to assess the total salivary microbial load between them.

2. Materials & Methods

The patients for this study were selected from the outpatient section of Department of Periodontology, Rajarajeswari Dental College and Hospital, Bangalore. Patients of both genders who fulfilled the following criteria were considered for the study.

A total of 45 subjects were evaluated in the study and were categorized into following groups.

Group I: 15 subjects with chronic periodontitis alone.
Group II: 15 subjects with chronic periodontitis and uncontrolled type 1 diabetes.
Group III: 15 subjects with chronic periodontitis and uncontrolled type 2 diabetes.

Periodontitis patients were included based on the clinical and radiographic assessment. Only patients with untreated chronic periodontitis with a probing depth ≥5mm, Clinical attachment loss ≥3mm, and radiographic evidence of alveolar bone loss on atleast two teeth per quadrant excluding the third molars were selected for the study.The periodontal status of all the patients was assessed using Russell's index.

Patients in diabetic groups were selected based on theglycatedhaemoglobin level(HbA1c) HbA1c ≥7%. Diagnosis of the type of diabetes was confirmed by the physician.

Salivary samples were collected from each subject. Unstimulated saliva was collected in a disposable cup, and immediately following that pH strips were used to measure the salivary pH.

2ml of pooled unstimulated saliva in the vestibule was collected using a syringe; transferred to a vial containing peptone water solution and carried immediately to the lab. It was then cultured on chocolate and blood agar medium and assessed for the microbial load.

3. Statistical Analysis

The obtained values were tabulated and subjected to statistical analysis. The data were analyzed using Statistical Package for the Social Sciences (SPSS 19) software. Mean, Standard deviation, Standard error and coefficient of variation of pH levels on three groups (control, DM Type 1 and DM Type 2) were assessed. Pair-wise comparison between the groupswith respect to pH levels by Tukeys multiple posthoc procedures. Comparison of the three groupswith frequency of appearance of different microbial species were done using Chi- Square Test.

4. Results

The salivary pH values of subjects in Group I i.e control group had a mean value of 7.47 ± 0.30. Similarly, salivary pH values of subjects in Group II i.e type I diabetes group had a mean of 6.73 ± 0.26, and salivary pH values of subjects in Group III i.e type II diabetes group had a mean of 7.27 ± 0.26 (Table 1).

Intra group comparison of the salivary pH among different groups using Tukeys multiple posthoc procedures revealed that the mean pH of saliva in group II (6.73) was significantly less than that of the group I (7.47) and group III (7.27). While there was no statistically significant difference was noted among group Group I and Group III. (Table 2, Graph 1)

The results showed that the pH of saliva in periodontitis patients with type 1 daibetes was more acidic compared to that of periodontitis patients with type II diabetes and Patients with periodontitis alone.

The species observed in the culture where divided based on the frequency of their appearance among different groups. The results showed that microbial count for streptococcus species was more in group II compared to group I and III but failed to show any statistical significant difference. Microbial count for Klebsiella, Pseudomonas and E.coli were more in group I compared to the other groups with no statistically significant difference among the groups. While group III showed more appearance of Moraxella, Staphylococcus and micrococi. Statistical significant difference was presentin frequency of appearance of micrococci species in group III compared to that of the other groups.

5. Discussion

Diabetes has emerged as a common and widespread health care problem in the world, which affects multiple organ systems. Even the oral cavity show changes related to the disease. Oral manifestations of diabetes mellitus includes dental caries, salivary dysfunction, oral mucosal and other oral infections, taste and neurosensory disorders, gingivitis, periodontitis etc.[15]

The present study evaluates the salivary pH in type I & II diabetes patients with periodontitis compared to patients with periodontitis alone. In the oral cavity, the pH is maintained near neutrality by saliva. The saliva maintains the pH by two mechanisms. Firstly the salivary flow eliminates the carbohydrates which could be metabolized by the bacteria hence the acid produced by the bacteria is removed. Secondly, the buffering activity of saliva neutralizes the acidity.[4]In the present study un-stimulated saliva samples were collected to assess the salivary pH from the patients as the composition and pH may alter in stimulated salivary samples.

In our study the mean salivary pH was shown to be significantly more acidic in type I diabetes patients with periodontitis compared to the other groups (Table 2, Graph 1). The pH of saliva is maintained by carbonic acid and

bicarbonate system, phosphate system and protein system of buffers. Acidic pH was also observed in studies conducted by *M E Lopez* et al. (2003) and *Prathibha K.M* et al. (2013) where it was attributed to either the microbial activity or a decrease in bicarbonate, which had occurred along with the flow rate.[2, 16]A study by *A.R Moreira* et al. (2009) states that the low saliva pH of type I diabetes patients is strong evidenceof reduced buffering capacity of the saliva and low flow of the unstimulated saliva.[17]

Low salivary pH promotes the growth of aciduric bacteria which then allows the acidogenic bacteria to proliferate creating an inhospitable environment for the protective oral bacteria. This allows for a shift in the oral environmental balance to favour cariogenic bacteria, which further lowers the salivary pH and the cycle continues.[18] According to the study by *Takahashi* et al (1990,1997) the periodontal pathogens grow favourably in an acidic environment ranging from 5.0-7.0.[12, 13] This signifies that the lowered pH in type I diabetes patients would lead to more favourable environment for the growth of periodontal pathogens causing advanced periodontal destruction.

Microbial load was assessed to check if there is any difference in the salivary microbiota among the groups with type I & II diabetescompared to that of the periodontitis group. Various gram positive and gram negative cocci and rods were observed in the culture such as streptococci, staphylococci, Moraxella, Klebsiella, Pseudomonas, E. coli, Micrococci.

Micrococci was found to be statistically significant in its frequency of appearance in type II diabetes patients with periodontitis. Species of micrococci are generally considered asnon-pathogenic commensals that colonize the skin,mucosae and oropharynx.However, it is now recognized that Micrococcus spp. can be opportunistic pathogens in the immuno-compromised patients.[19] Study by *R.Pece*set al (1997) showed that Micrococcus luteus caused relapsing bacteremia in an immune-compromised individual.[19]A study done by *IwaiT (2009)*showed that periodontal disease also leads to bacteremia causing vascular diseases.[20] As uncontrolled diabetes is known to cause an immunocompromised state, it may lead to increased risk of opportunistic infection by micrococci to cause bacteremia. In our study the results showed significant increase in the micrococci species in patients with type II diabetes with periodontitis. This indicates an overall increased risk of bacteremia by periodontal disease as well as micrococci in patients with type II diabetes mellitus.

6. Conclusion

Since diabetes allows prolonged periods of hyperglycemia to begin exerting negative effects on various organ systems including oral cavity. Acidic pH favours growth of periopathogens. Hence adequate measures to prevent periodontitis in patients at an early stage are necessary. Understanding the effects of diabetes mellitus on the oral health is, therefore, must for the dental professionals.

Diabetes causes an immunocompromised state favoring various micro-organisms to cause opportunistic infections. Further studies have to be carried out to understand the link between diabetes, periodontal disease and micrococci and to know their role in bacteremia.

7. Source of Funding
None

8. Conflict of Interest
None

9. Tables & Graphs

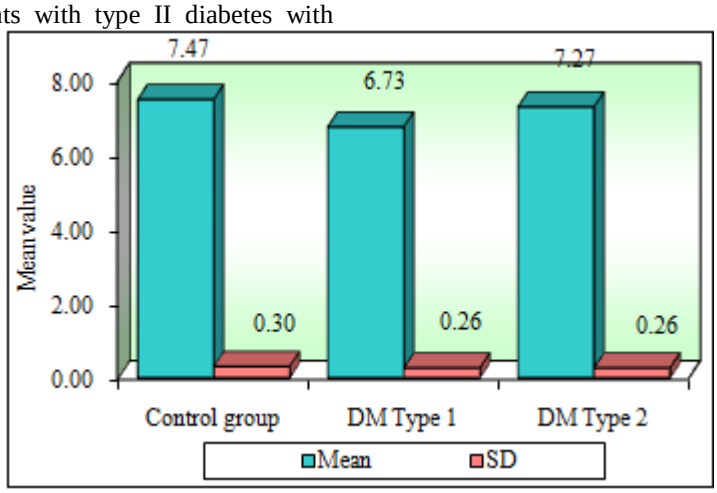
Table 1: Mean, SD, SE and coefficient of variation of pH levels on three groups (control, DM Type 1 and DM Type 2)

Groups	Mean	SD	SE	CV
Control group	7.47	0.30	0.08	3.98
DM Type 1	6.73	0.26	0.07	3.83
DM Type 2	7.27	0.26	0.07	3.55

Table 2:Pair-wise comparison between the groupswith respect to pH levels by Tukeys multiple posthoc procedures

Variable	N	Mean	P value
GROUP 1	15	7.47	0.0001*
GROUP 2	15	6.73	
GROUP 1	15	7.47	0.1209
GROUP 3	15	7.27	
GROUP 2	15	6.73	0.0001*
GROUP 3	15	7.27	

*p<0.05



Graph 1: Comparison of three groups (control, DM Type 1 and DM Type 2) with respect to pH levels

Table 3: Comparison of three groups (control, DM Type 1 and DM Type 2) with frequency of appearance of different microbial species

Organisms	Groups	Appearance	%	No Appearance	%	Total
Moraxella	Group 1	4	26.67	11	73.33	15
	Group 2	4	26.67	11	73.33	15
	Group 3	7	46.67	8	53.33	15
	Chi-square=1.8001 P = 0.4071					
Streptococcus	Group 1	7	46.67	8	53.33	15
	Group 2	10	66.67	5	33.33	15
	Group 3	4	26.67	11	73.33	15
	Chi-square=4.8221 P = 0.0901					
Micrococci	Group 1	0	0.00	15	100.00	15
	Group 2	0	0.00	15	100.00	15
	Group 3	3	20.00	12	80.00	15
	Chi-square=6.4219 P = 0.0402*					
Klebsiella	Group 1	1	6.67	14	93.33	15
	Group 2	1	6.67	14	93.33	15
	Group 3	0	0.00	15	100.00	15
	Chi-square=1.0472 P = 0.5932					
Staphylococcus	Group 1	0	0.00	15	100.00	15
	Group 2	0	0.00	15	100.00	15
	Group 3	1	6.67	14	93.33	15
	Chi-square=					
Pseudomonas	Group 1	1	6.67	14	93.33	15
	Group 2	0	0.00	15	100.00	15
	Group 3	0	0.00	15	100.00	15
	Chi-square=2.0452 P = 0.3601					
E.Coli	Group 1	2	13.33	13	86.67	15
	Group 2	0	0.00	15	100.00	15
	Group 3	0	0.00	15	100.00	15
	Chi-square=4.1862 P = 0.1231					

*p<0.05

References

[1] Meenawat A, Punn K, Srivastava V, Meenawat AS, Dolas R S, Govila V. Periodontal disease and type I diabetes mellitus: Associations with glycemic control and complications. J Indian SocPeriodontol 2013;17:597-600
[2] K M P, Johnson P, Ganesh M, Subhashini AS. Evaluation of salivary profile among adult type 2 diabetes mellitus patients in South India. J ClinDiagn Res 2013;7:1592-5
[3] American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care2010; 33(Suppl 1):S62–9.
[4] Seethalakshmi C., Jagat Reddy R. C., Asifa N., Prabhu S. Correlation of salivary pH, incidence of dental caries and periodontal status in diabetes mellitus patients: A cross-sectional study. Journal of Clinical and Diagnostic Research. 2016;10(3):ZC12–ZC14.
[5] Taylor GW. Bidirectional interrelationship between diabetes and periodontal diseases: Anepidemiologic perspective. Ann Periodontol. 2001;6:99–112.
[6] Loe H. Periodontal disease. The sixth complication of diabetes mellitus. Diabetes Care.1993; 16:329–34.
[7] Cianciola LJ, Park BH, Bruck E, Mosovich L, genco RJ. Prevalence of periodontal disease in insulindependent diabetes mellitus (juvenile diabetes) J Am Dent Assoc. 1982; 104:653–60.
[8] Nelson RG, Shlossman M, Budding LM, Pettitt DJ, Saad MF, Genco RJ, et al. Periodontal diseaseand NIDDM in Pima Indians. Diabetes Care. 1990; 13:836–40.
[9] Thorstensson H, Hugoson A. Periodontal disease experience in adult long duration insulin dependentdiabetics. J ClinPeriodontol. 1993; 20:352–8.
[10] Grossi SG, Genco RJ. Periodontal disease and diabetes mellitus; two way relationship. AnnPeriodontol. 1998; 3:51–61.
[11] Donnahue R, Wu T. Insulin resistance and periodontal disease: An epidemiologic overview ofresearch needs and future directions. Ann Periodontol. 2001; 6:199–224.
[12] Takahashi N, Schachtele CF. Effect of pH on the growth and proteolytic activity of Porphyromonasgingivalis and Bacteroidesintermedius. J Dent Res.1990;69:1266–69
[13] Takahashi N, Saito K, Schachtele CF, Yamada T. Acid tolerance and acid neutralizing activity of Porphyromonasgingivalis, Prevotellaintermedia and Fusobacteriumnucleatum. Oral MicrobiolImmunol. 1997; 12:323–28.
[14] Kulshrestha, R., Neral, A., Srinivasa, T.S. and Baig, S.A. Comparison of oral microflora of diabetic and non-diabetic patients with periodontitis. J. Pure Appl. Microbiol., 2011; 5: 883-886.
[15] Bangash RY, Khan AU, Tariq KM, Yousaf A. Oral aspects and complications in type 2 diabetes mellitus – a study. Pakistan Oral & Dental Journal. 2012; 32(2):296-99.
[16] López ME, Colloca ME, Páez RG, Schallmach JN, Koss MA, Chervonagura A. Salivary characteristics of diabetic children. Brazilian Dental Journal. 2003; 14(1):26-31.
[17] Moreira A.R., Passos I.A., Sampaio F.C., Soares M.S.M., Oliveira R.J. Flow rate, pH and calcium concentration of saliva of children and adolescents with type 1 diabetes mellitus.Braz J Med Biol Res. 2009; Aug; 42(8): 707-711.
[18] Goyal D, Kaur H, Jawanda MK, et al. Salivary pH and dental caries in diabetesmellitus. International Journal of Oral & Maxillofacial Pathology. 2012; 3(4):13-16.
[19] Peces R et al. Relapsing bacteraemia due to Micrococcus luteus in a haemodialysis patient with a Perm-Cath catheter.Nephrol Dial Transplant. 1997 Nov;12(11):2428-9
[20] Iwai T. Periodontal bacteremia and various vascular diseases. J Periodont Res 2009;44: 689–694.

Author Profile

Dr Mohammed Salman, BDS, (MDS), Post Graduate, Department of Periodontology, Rajarajeswari Dental College & Hospital, Bangalore

Savita Sambashivaiah, MDS, Professor & Head Of The Department, Department of Periodontology, Rajarajeswari Dental College & Hospital, Bangalore