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ORIGINAL RESEARCH

Evaluation of Subgingival Microflora in Diabetic and Nondiabetic Patients

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ABSTRACT

The present investigation was designed with the aim of studying the microbiota of diabetic patients—both insulin dependent and noninsulin dependent and nondiabetic individuals. Each of the three groups had 15 patients, coming under the age group of 35 to 55 years and all having periodontitis.

Even though the microbial flora are almost the same, specific microorganisms may not be predisposing cause for the periodontal disease process in diabetics.

The study is clinically significant by means of its implication in the treatment of bacterial infections related to periodontitis and in those patiets who are having systemic diseases, like diabetes along with poor periodontal condition and infections.

Keywords: Microflora analysis, IDDM, NIDDM, Nondiabetic.

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INTRODUCTION

Diabetes mellitus is a clinical syndrome characterized by the hyperglycemia due to absolute or relative deficiency of insulin.[4] It is a heterogenous primary disorder of carbohydrate metabolism, with multiple etiologic factors that generally involve absolute or relative insulin deficiency, or insulin resistance or both, ultimately leading to persistent hyperglycemia which is the hallmark of this disease syndrome.[18]

We are dealing with a polymicrobial, multifactorial disease which therefore have many interactive risk factors. It is thus to be expected that a wide distribution of susceptibility to the disease may be demonstrable in any population.[8]

Periodontal disease is a common infection-induced inflammatory disease among individuals suffering from diabetes mellitus.[6] Periodontal diseases are microbial in origin. A large population of these organisms are gram-negative microaerophilics or anaerobic bacteria. Among the systemic factors influencing the etiopathogenesis of periodontal disease is diabetes mellitus, which is one of the major health problems.

Newman et al believed that specific types of bacteria are responsible for the destruction of periodontal support and ultimate tooth loss in various forms of periodontal disease. Characteristic types of microflora are associated with different clinical patterns of periodontal disease (Socransky 1977).

Thus, it is of interest to note the periodontal destruction in diabetes and adult periodontitis, and find any correlation that might exist with specific microorganisms in the subgingival microflora. Bacterial infections pose a special problem in cases of diabetes because these patients often exhibit depressed host defense and repair mechanisms that may be either intrinsic or secondary to loss of diabetic control. Similarly, several previous studies indicate that diabetes mellitus may influence the prevalence and severity of periodontal disease.[14]

AIMS AND OBJECTIVES

1. To evaluate the subgingival microflora in diabetes mellitus (IDDM and NIDDM) and nondiabetic patients. Both having adult periodontitis.
2. To find out the prevalence of different microorganisms in the subgingival environment of both diabetic and nondiabetic patients.
3. To identify the predominance of different types of micro organisms in diabetic and nondiabetic patients.
4. To compare the microflora in diabetic and nondiabetic individuals.

Periodontal Disease and Diabetes

The incidence of periodontitis increases among diabetic patients. Periodontal disease may be more severe in diabetics with advanced systemic complications.[12]

It has been proved that IDDM patients have and increased risk of developing periodontal disease with age. Under similar conditions of plaque control, adult subjects with IDDM had lost more approximal attachment, bone, than well-controlled diabetic subjects.

Studies conducted on Pima Indians suffering from NIDDM proved that irrespective of age, subjects with diabetes, had a higher prevalence of periodontal disease using either periodontal attachment loss, or radiographic bone loss. Compared to nondiabetic individuals, subjects with NIDDM were 2.8 times more likely to have periodontal disease defined by clinical attachment loss, and 3.4 times more likely defined by radiographic bone loss.

Bacterial Associations in Diabetes

Induction of experimental diabetes in rats is known to cause a shift in subgingival bacteria to a periodontopathic flora predominated by Gram-negative rods and filaments, with subsequent deepening of periodontal pockets.

Capnocytophaga species predominate in most periodontal lesions of IDDM patients, averaging 24% of the cultivable flora in one report.[19] *Actinobacillum actinomycetemcomitans* was found in cultures of the subgingival flora in three out of nine subjects with IDDM.[19] Black pigmented *Bacteroides* and *Fusobacterium* species comprised a small percentage of the periodontal isolates.[19]

The composition of periodontal microflora found in periodontally diseased sites of NIDDM patients appears similar to that found in chronic adult periodontitis. *Prevotella intermedia*, *Campylobacter rectus*, and *Porphyromonas gingivalis* have been found as the most predominant pathogens in subgingival dental plaque of NIDDM patients.[19] Around 67 to 88% of the patients were positive for these species. Immunofluorescent microscopic examination revealed that *A. actinomycetemcomitans* was present in small numbers. Higher levels of *P. intermedia* have been reported in diseased *vs* healthy periodontal sites in IDDM.

MATERIALS AND METHODS

A total of 45 patients were selected for this study. Divided into three groups of 15 patients each. The patients belonged to the age category of 35 to 55 years and having periodontitis.

- Group I—insulin-dependent diabetes mellitus patients
- Group II—noninsulin-dependent diabetes mellitus
- Group III—adult periodontitis patients without diabetes

Criteria for Selection

There was no history of other systemic disorders, none had received antibiotics, oral antiseptics, or conventional periodontal treatment in the preceding 6 months.

The blood sugar level of selected patients were standardized as 120 to 130 mg/dl fasting and 250 to 300 mg/dl postprandial. Uncontrolled diabetes patients were excluded.

The oral cavity is examined for periodontal pockets using Williams graduated probe. Only pockets 4 to 7 mm deep were considered. Random selection of pockets were done. The sample site was first wiped clean to remove supragingival plaque and debris. The area is then isolated using rolls and air-dried. Now standardized no. 30 paper point is inserted to the depth of the pocket until resistance is felt.

After 30 seconds, the paper point is removed and immediately inserted into the Robertson cooked meat transport medium in a test tube.

Another paper point is then inserted into the same periodontal pocket, and soaked. This is removed and a smear is made on a sterile glass slide. The slide is then air-dried. This will be used for Grams stain procedure.

Microbiological Procedure

Plaque samples are transported to the lab in prereduced Robertson cooked meat (RCM) media, used as transport fluid. Bacteria were dispersed by mixing with a vortex mixer for 60 seconds an inoculating loop of 4 mm is used to collect 0.01 ml of fluid, and is spread onto the culture plates. This method is termed as 'Standard loop semiquantitative method'.

The culture plates used in this case were enriched trypticase soya agar (ETSA) for total cell counts, and tryptic soya agar plus bacitracin, vancomycin and horse serum (TSBV) for isolation of *Aa comitans*.

Tryptic soya agar[1] is a general purpose media used for cultivation of a wide variety of aerobic and anaerobic bacteria.

After culturing by incubating the plates in an anaerobic jar for 48 hours, colony forming units are counted.

Bacteria were primarily identified by colony morphology, Gram stain and oxidase activity, biochemical reactions using disks of dextrose, maltose, lactose, sucrose, sorbitol, cellobiose and ornithine.

For this, cystine tryptose agar (CTA) medium is used. Bacteria from the cultured plates are placed on to the CTA and then the carbohydrate disks are placed. Particular organism will ferment only a particular sugar. So, on the change of color of the medium to yellow, fermentation is detected. The organism is identified in relation to the disk placed.

Aa comitans was primarily identified by colony morphology-star shaped inner structure or ridges-cell morphology, catalase and oxidase production and sugar fermentation.

Statistical Analysis

Comparison among the three groups was found by a non-parametric test called 'Kruskel-Wallis test'.

Comparison of the insulin-dependent patient group *vs* nondiabetic group and noninsulin-dependent patient group versus nondiabetic group is calculated using 'Mann-Whitney U test'.

RESULTS

From this study, it is clear that the following microorganisms are associated with the disease process in the insulin-dependent, noninsulin-dependent and nondiabetic patients within an age group of 35 to 55 years, and all three groups having periodontitis.

A total of 45 patients were examined during the study, werein subgingival plaque samples were collected and cultured to find out the different organisms.

Group I: Insulin-Dependent Diabetics

Microorganism	Presence in percentage of patients
Peptostreptococcus	60.0
Bacteroides	46.7
A. actinomycetemcomitans	66.7
P. gingivalis	53.3
Capnocytophaga	13.3
S. sanguis	53.3

Group II: Noninsulin-Dependent Diabetics

Microorganism	Presence in percentage of patients
Peptostreptococcus	46.7
Bacteroides	33.3
Aa comitans	73.3
P. gingivalis	66.7
Fusobacterium	6.7
S. sanguis	46.7
P. intermedia	46.7
B. fragilis	13.3

Group III: Nondiabetics

Microorganism	Presence in percentage of patients
Peptostreptococcus	26.7
Bacteroides	26.7
Aa comitans	73.3
P. gingivalis	33.3
Fusobacterium	6.7
S. sanguis	66.7
P. intermedia	53.3
C. rectus	6.7
B. fragilis	13.3

IDDM *vs* Nondiabetic

Organism	*Test statistics 'Z'*	*Remarks*
Peptostreptococcus	1.867	p > 0.05
Bacteroides	0.477	p > 0.05
A. actinomycetemcomitans	1.125	p > 0.05
P. gingivalis	1.245	p > 0.05
S. sanguis	1.458	p > 0.05
P. intermedia	1.346	p > 0.05
B. fragilis	1.576	p > 0.05

Result shows no statistically significant difference between the two compared groups.

NIDDM *vs* Nondiabetic

Organism	*Test statistics*	*Remarks*
Peptostreptococcus	1.493	p > 0.05
Bacteroides	0.549	p > 0.05
A. actinomycetemcomitans	0.478	p > 0.05
P. gingivalis	1.804	p > 0.05
Fusobacterium	0.185	p > 0.05
S. sanguis	1.125	p > 0.05
P. intermedia	0.848	p > 0.05
C. rectus	0.124	p > 0.05
B. fragilis	1.116	p > 0.05

There is no statistically significant difference between the groups compared.

IDDM *vs* NIDDM

Organism	*Test statistics 'Z'*	*Remarks*
Peptostreptococcus	1.285	p > 0.05
Bacteroides	1.108	p > 0.05
A. actinomycetemcomitans	1.495	p > 0.05
P. gingivalis	2.020	p < 0.05
S. sanguis	1.540	p > 0.05
P. intermedia	1.080	p > 0.05

There is significant difference at 5% level in case of *P. gingivalis*, when IDDM and NIDDM groups were compared.

Predominant Microorganisms

Organism	*Group I IDDM*	*Group II NIDDM*	*Group III AP-nondiabetic*
P. gingivalis	53.0	66.7	33.3
P. intermedia	46.7	6.7	53.3
A. actinomycetemcomitans	66.0	73.3	73.3
Peptostreptococcus	60.0	46.7	26.7
S. sanguis	53.3	46.7	66.7
Bacteroides	46.7	33.3	26.7

DISCUSSION

It has been proved beyond doubt that periodontal disease is of microbial origin. Host response can be altered by a multitude of factors like systemic disease, hormonal, nutritional, genetic factors and senility. Diabetes is one major disease which influences the periodontal health. Its related to increased severity of periodontitis.

Microbial succession and change in microbial composition from tooth to tooth or even surface to surface have been attributed to the following factors: Selective bacterial adherence, bacterial synergism and antagonism, oxygen tension, pH of the plaque, thickness of the plaque, availability of nutrients and host-bacterial factors.

In the present study, among the three groups examined, diabetic and nondiabetic patients having adult periodontitis, the microbial flora did not show statistically significant difference.

The whole group comparison was done using 'Kruskal-Wallis test', and intergroup comparison was done using 'Mann-Whitney U test'.

STUDIES

Scoop observed that local factors causing periodontal disease elicit a greater response in patients with uncontrolled diabetes, but there is no type of oral lesion, that is characteristic or pathognomonic of diabetes.

Studies by Zambon[20] suggested the following as microorganisms associated with periodontal disease of adult periodontitis and periodontitis in diabetic patients.

Adult Periodontitis

- *Actinobacillus actinomycetemcomitans*
- *Bacteroides intermedius*
- *Bacteroides gingivalis*
- *Bacteroides forsythus*
- *Capnocytophaga gingivalis*
- *Eikinella corrodens*
- *Eubacterium species*
- *Fusobacterium nucleatum*
- *Propionibacterium acnes*
- *Streptococcus intermedius*
- *Wolinella recta*

Periodontitis in Insulin-Dependent Diabetes

- *A. actinomycetemcomitans*
- Anaerobic vibrios
- Campylobacter
- *Capnocytophaga*

Periodontitis in NIDDM

- *Bacteroides gingivalis*
- *Bacteroides intermedius*
- *Fusobacterium species*
- *Wolinella recta*

There is no longer any doubt that Gram-negative bacteria play a major role in the pathogenesis of human periodontal diseases (Socransky 1977, Slots 1979). *Bacteroides gingivalis, Bacteroides intermedius*, and *Aa comitans* occupy predominant positions in advancing periodontitis, because of their high frequency of isolation and pathogenic potential (Slots 1986).

Other periodontal pathogens include *Wolinella recta, Bacteroides forsythus, Eikinella corrodens*, and some *Fusobacterium* and *Treponema* species.[15]

Slots and Listgarten (1988) suggested that *Bacteroids gingivalis, Bacteroides intermedius* and *A.actinomycetemcomitans*, seem to be major pathogens in advancing periodontitis in man. Antibody levels against *B. gingivalis* and *A. actinomycetemcomitans* are markedly elevated in serum and gingival crevicular fluid of periodontitis patients.

In 1991,Culter, van Dyke et al studied PMNL function in adult IDDM patients. There was decreased chemotaxis and phagocytic functions and increased superoxide production. The bacterial species recovered from the adult IDDM patients included *P. gingivalis, A. actinomycetemcomitans, Peptostreptococcus micros* and *E.corrodens*. These flora appear to represent a combination of that found previously in juvenile IDDM patients, and that found in adult NIDDM patients.

Classic diabetic periodontitis is evidenced by a widening of periodontal ligament space, a suppurative exudate, deep periodontal pockets, multiple lateral periodontal abscesses and marked alveolar bone resorption.[2]

Mandel et al (1992) studied group of poorly controlled IDDM patients for microbial levels, microbial incidence and their percentage levels. Increased levels of *P. intermedia, P. melaninogenica* species, *Bacteroides gracilis, Eikinella corrodens, Fusobacterium nucleatum* and *Campylobactor rectus* were found at the periodontally diseased sites.

According to the position paper published in Journal of Periodontology (1996;67:166-76). Diabetes and periodontal diseases, the composition of periodontal microflora found in periodontally diseased sites of NIDDM of patients appears to be similar to that found in chronic adult periodontitis. *P. intermedia, C. rectus*, and *P. gingivalis* have been found as the three most predominant pathogens in subgingival dental plaque of NIDDM patients.[13]

A higher prevalence of *P. gingivalis* was noted in type II diabetes, when compared to nondiabetics, demonstrated through polymerization chain reaction.[17]

Smith et al (1996) studied microflora in IDDM patients. *A. actinomycetemcomitans* were never detected in the 18 patients studied. *P.gingivalis* was present at 7% of the sites both before and after treatment. *B. forsythus* was present at 29% of sites before and 36% of sites after treatment. Positive associations were found between the presence of *B. forsythus* and AST (aspartate amino tranferase) values, gingival index, probing depth and attachment level.[16]

Several studies have investigated the composition of plaque in diabetics when compared to nondiabetics. Increased number of so-called periodontal pathogens have been isolated from periodontal pockets of diabetic patients.[5]

Nakou et al[10] studied microflora in adult periodontits and named *P. intermedia, P. gingivalis, Aa comitans*, as dominant pathogens, and also sited presence of *C. rectus* and *B. forsythus*.

The result of the present study is also in line with the above-mentioned studies. In the three groups studied, IDDM, NIDDM and nondiabetic patients, all having adult periodontitis, the microflora did not show statistically significant difference.

Thorstensson et al observed significantly greater number of *P. gingivalis* in diabetics when compared to control, although no difference was seen with *Aa comitans, C. rectus, Capnocytophaga, E. corrodens, F. nucleatum* and *P. intermedia*.[3]

The predominant organisms of IDDM group were found to be *Aa comitans* (66.7%), followed by *Peptostreptococcus* (60%), *P. gingivalis* (53.3%), *S. sanguis* (53.3%) and *P. intermedia* isolated from 46.7% of the patients.

In NIDDM group, the predominant flora were, *Aa comitans* being isolated from 73.3% of the patients of the group, *P.gingivalis* (66.7%), *P. intermedia* (46.7%), *Peptostreptococcus* (46.7%) and *S. sanguis* (46.7%).

The nondiabetic adult periodontitis group had predominance of *Aa comitans* (73.3%), *S. sanguis* (66.7%), *P. intermedia* (53.3%) and *P. gingivalis* (33.3%).

There was presence of *Capnocytophaga* (13.3%) in group I (IDDM), *Fusobacterium* (6.7%) in group II (NIDDM) and *C. rectus* (6.7%) in group III (nondiabetic).

In a more recent study, periodontal microbiota of diabetic and nondiabetic patients were compared using checker board DNA-DNA hybridization. Of the 17 species tested for, *T. denticola, S. sanguis, P. nigrescens, Staph. intermedius, S. oralis, levels* were elevated in the supragingival plaque of diabetics, compared with nondiabetics, although no significant differences were found in subgingival samples.[7]

Subgingival infection patterns were also found similar in type I diabetes and nondiabetic controls of comparable periodontal status.[11]

Mealy et al showed significantly increased frequency of *P. gingivalis* campylobacter species and *A. actinomycetemocomitans* in the subgingival plaque of diabetics, when compared to nondiabetics.[9]

The inference of this study is that even though the microbial flora are almost the same, specific microorganisms may not be the predisposing cause for periodontal disease process in diabetics. It is the compromised host response and the systemic and tissue conditions in diabetic which make them more vulnerable to tissue destruction.

This study was conducted on a patient population of age between 35 and 55 years. Further studies can be done for the analysis and comparison of microflora of juvenile diabetics with insulin-dependent adult patients and noninsulin-dependent diabetics.

REFERENCES

1. Barbara JH. Clinical and pathogenic microbiology (2nd ed), Chapter 59, Page 909, Mosby Publishing Co.
2. Culter CW, Eke P, et al. Defective neutrophil function in insulin dependent diabetes mellitus patient. A case report. JP 1991; 62;394-401.
3. Dahlen G, Hintao J, Ratarasan C. The microbiological profiles of sub and supragingival plaque in adults with and without Type I diabetes. Oral Microbiol Immunol 2007;22:175-81.
4. Edwards CRW, Boucher IAD. Davidson's Principles and Practice of Medicine. P-724, (17th ed), Churchil Livingstone.
5. Ford PJ, Cullinan MP, Seymor GJ. Relationship between periodontal infection and systemic diseases. Clin Microbiol Infect 2007;13:3-10.
6. Grossi SG, Robertson DC. Treatment of periodontal disease in diabetes reduces glycated hemoglobin. JP 1997;68:713-19.
7. Genco R, Nelson RG, Hanson RL, Schlossman M. Periodontal disease in Type II diabetes. Diabetes care 2010;28(1):27-32.
8. Johnson NW. Risk markers for oral disease periodontal diseases. Preface Cambridge University Press 1991.
9. Mealy BL, Ebersole. Periodontal disease and diabetes. A two way street. J Am Dent Assn 2006;137 Suppl 2:26-31S.
10. Nakau PA, Manti F. Microflora in adult periodontitis. JPR 1995;30:325-33.
11. Offenbacker S, Moss K, Southerland JH. Commonality in chronic inflammatory diseases, periodontitis and diabetes. Periodontology 2000, 2006;40:130-43.
12. Plancak D. CPITN assessment of periodontal status in diabetic patients. J Perio 1988;59;816-22.
13. Position paper. Diabetes and periodontal diseases. JP 1996;67: 166-76.
14. Schlossman M, Knowler WC. Oral health comparison of diabetic and nondiabetic subjects. JDR 1985;288.
15. Slotts J, Listgarten MA. B. gingivalis, B. intermedius, and A. actinomycetemcomitans in human periodont al disease. JCP; 1988;15:85-93.
16. Smith GT, Johnson BD. Short-term response to periodontal therapy in IDDM patients. JP 1996;67:784-802.
17. Uzzazu S, Salem A, Campus G. Diabetes and periodontal diseases. A case control study. J Periodontol 2005;76:418-25.

18. Wyngaarden, Smith-Cecil. Textbook of medicine (17th ed), Igaku-Shoin/Saunders International Edn, P-1320.
19. Zambon JJ, Reynolds H. Microbiological and immunological studies of adult periodontitis in patients with non-insulin dependent diabetes melliyus. JP 1988;59:23-31.
20. Zambon JJ. Microbiology of periodontal disease. The nature of periodontal tissues in health and disease. Chapter 11; P 147.

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