Article published online: 2022-07-07

ARTICLE

Microbiology of Periodontitis in Diabetic Patients in Oran, Algeria

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Published: 01 September 2013
Ibnosina J Med BS 2013;5(5):280-287
Received: 24 November 2012
Accepted: 02 January 2013
This article is available from: http://www.ijmbs.org

Abstract

Background: Poorly controlled diabetes increases the risk of periodontitis. The link between diabetes and periodontal disease is increasingly recognized and that diabetics are more susceptible to periodontal disease. **Material and methods:** Cultures of sub-gingival plaque from 62 subjects suffering from aggressive periodontitis (37 patients with diabetes and 25 control cases) and 136 patients with chronic periodontitis (92 with diabetes and 44 control cases) were done on selective and nonselective media. The bacterial isolates were identified by conventional biochemical tests. **Results:** In aggressive periodontitis, *Actinomyces israelii* was present in patients with diabetes only while *Actinomyces naeslundii* was positive in the control group only. The rates of *Eubacterium nodatum, Capnocytophaga spp., Peptostreptococcus anaerobius*, and *Eikenella corrodens*. *Actinobacillus actinomycetemcomitans* were remarkably present in diabetic patients. Culture of *Prevotella intermedia*, *Eubacterium spp* and *Campylobacter spp* were more remarkable in control patients with aggressive periodontitis. On the other hand, in chronic periodontitis, *Eikenella corrodens, Aggregatibacter aphrophilus,* and *Eubacterium* were more noticeable in patients with diabetes. **Conclusions:** Periodontal pathogens are distinctly different in patients with diabetes from those without diabetes. The differences are evident in both aggressive and chronic periodontitis. These different microbiological characteristics are relevant in both dental and general medical practices.

Key words: Aggressive periodontitis, chronic periodontitis, diabetes, periodontal pathogen, sub-gingival.

Introduction

Recent studies in periodontal medicine suggest that there is a link between mild to moderate periodontal disease in humans and certain systemic disorders such as diabetes mellitus, pneumonia, heart disease and preterm birth (1,2).

An association between periodontal disease and heart disease, birth of low weight, and type 2 diabetes mellitus has been established (3). Furthermore, recent studies have revealed that a bidirectional relationship is evident between diabetes mellitus and periodontal disease (1). Periodontal disease and diabetes are strongly interrelated and have common pathobiology. Inflammatory events during periodontal disease may play an important role in the development of diabetes and insulin resistance probably facilitates the progress of periodontal disease. Diabetes predisposes patients to oral infection, and once the infection is established, it exacerbates diabetes-related hyperglycemia and may even predispose otherwise healthy individuals to develop diabetes (4). Periodontal disease has been proposed as the sixth complication of both type 1 and type 2 diabetes (5,6). Solid data have corroborated the hypothesis that periodontal diseases are more prevalent among diabetics than non-diabetics (7,8) and that periodontitis is more severe in diabetic patients (9). Prevention and control of periodontal disease should be considered an integral part of diabetes control (8). Similarly, two studies were followed that provided additional evidence to support the assumption that severe periodontitis increases the risk of poor glycemic control (1). Limited data exist from newly developed and developing parts of the world on oro-dental health in diabetes. Therefore there is a pressing necessity to establish the profile of oral microbial infection to form a scientific basis to inform and guide oral health, general medical, and public health practices to emphasize the role of routine health care for people with diabetes. To this end, the present study was undertaken to establish the microbiological patterns of patient with aggressive and chronic periodontitis in patients with diabetes compared with those without diabetes.

Materials and Methods

Study Population

One hundred and ninety-eight patients aged 14-35 years with periodontitis who presented in the Department of Periodontology (Oran, Algeria) were divided into aggressive and chronic periodontitis groups according to the classification of Armitage *et al*. (10). Of these 198 cases, 62 suffered from aggressive periodontitis (37 patients with diabetes and 25 control cases) and 136 patients from chronic periodontitis (92 with diabetes and 44 control cases). Diabetes was diagnosed on basis of a random plasma glucose level of >200mg/dl or fasting glucose plasma level of > 140 mg /dl (11).

Sampling

After removal of supragingival plaque by means of sterile cotton balls, sub-gingival plaque was collected on Gracey curette inserted to the depth of the periodontal space (12,13). The specimens were processed within 2 h of sampling.

Bacteriological tests

Gram smears were done for each of the specimens. All specimens were analyzed and grown in appropriate culture media. Samples were collected and placed in the brain-heart broth for enrichment, and incubated for 2-4 h. From the broth culture, each sample was cultured on a variety of agar media including Trypticase soy agar with serum horse + Bacitracin and Vancomycin, TSBV (14), Bacteroides bile esculin agar (15) and Non-selective agar media such as blood agar, chocolate agar and MacConkey agar (16,17) and mannitol salt agar (16). Finally Sabouraud agar allowed the isolation of Candida. Cultures were incubated, at 37°C for 18-24 hours, both aerobically and anaerobically using anaerobic jar (Oxoid Ltd., Basingstoke, UK). Classification and identification of aerobic and anaerobic microbes were done according to standard routine techniques proposed previously (18). Bacterial isolates were identified by conventional biochemical tests.

Table 1. Relationship between periodontitis and diabetes diseases.

	Acute periodontitis		Chronic periodontitis	
	N	%	N	%
Diabetics	37	18.7	92	46.5
Group control	25	12.6	44	22.2

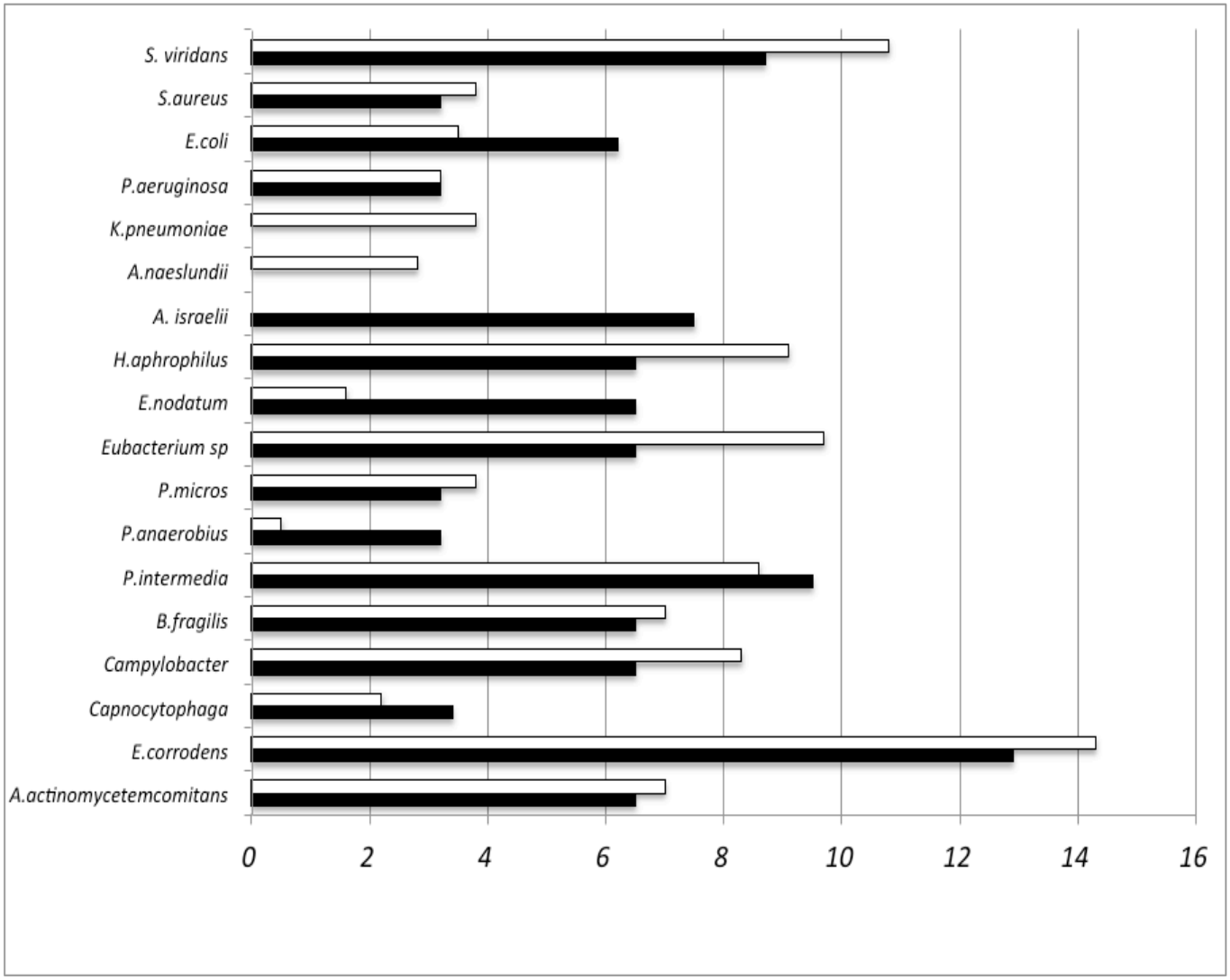

Figure 1. Distribution of periodontal bacteria in aggressive periodontitis in patients with diabetes (Solid) and those without diabetes (Open).

Statistical analysis

The statistical analysis was performed using SPSS Statistics 15.0 (IBM Inc., Chicago, IL, USA). Subject characteristics (age, number and site of periodontal pockets with PD ≥ 5 mm) and clinical diagnosis (aggressive or chronic periodontitis) were compared between diabetes and non-diabetes using Student-t test. For microbiologic results, the percentages of sites with organisms were compared between the diabetes and non-diabetes groups applying the Chi-square test.

Results

One hundred and ninety eight patients were diagnosed with periodontitis. Of these, 62 were suffering from aggressive periodontitis including 37 patients with diabetes (18.7%) and 136 patients with chronic periodontitis including 92 (46.5%) with diabetes (Table 1). The bacterial culture both aerobically and anaerobically in patients with aggressive periodontitis showed that *Actinomyces israelii* was present only in patients with diabetes while *A. naeslundii* and *Klebsiella pneumoniae* were positive only in non-diabetic patients. The rates of *Eubacterium nodatum, Escherichia coli, Prevotella intermedia, Capnocytophaga spp*. and *P. anaerobiu* were remarkably notable in diabetic patients. Cultures in non-diabetic patients showed that, *Eubacterium spp, Eikenella corrodens, H. aphrophilus, B. fragilis*, and *Campylobacter spp* were more prevalent. Distribution of periodontal bacteria in aggressive periodontitis in patients

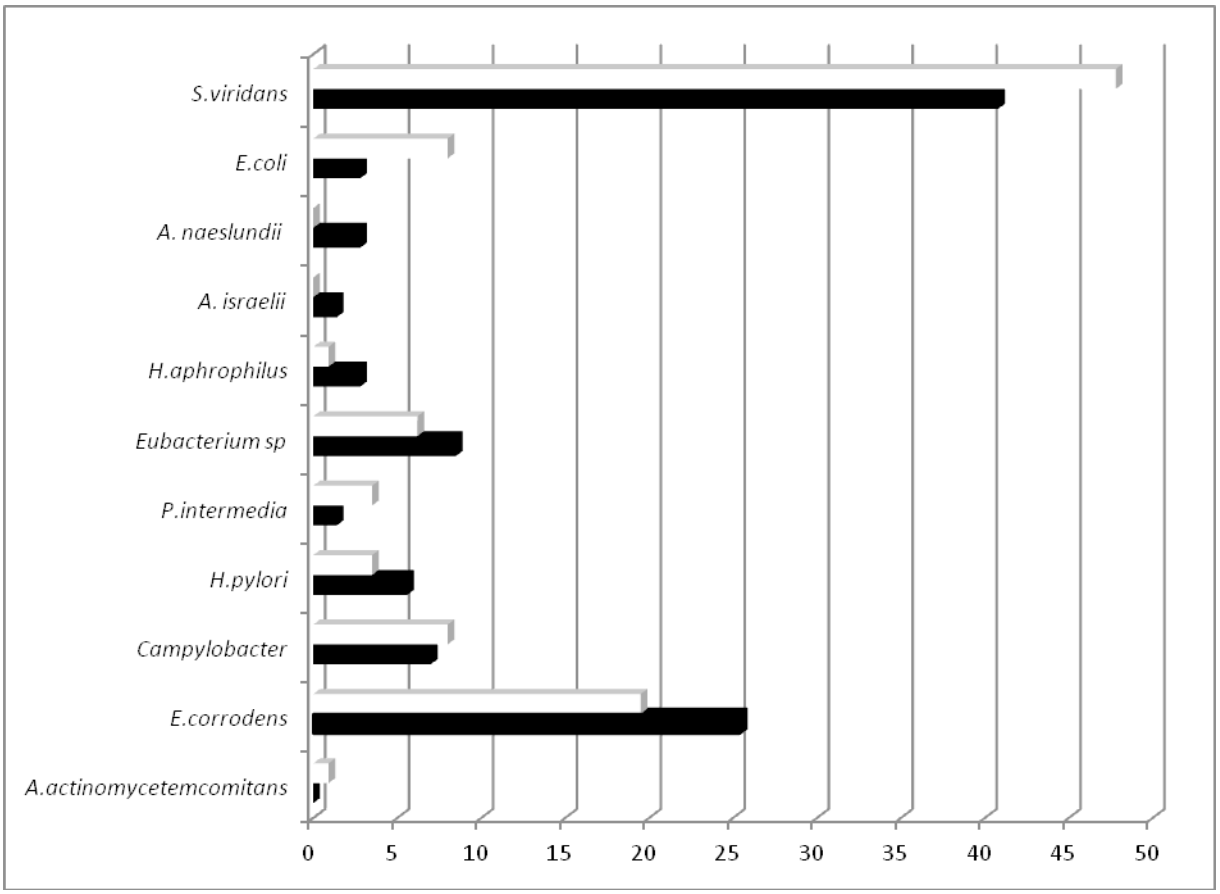

Figure 2. Distribution of periodontal bacteria in cases of chronic periodontitis in diabetic (Solid) versus non-diabetic patients (Open).

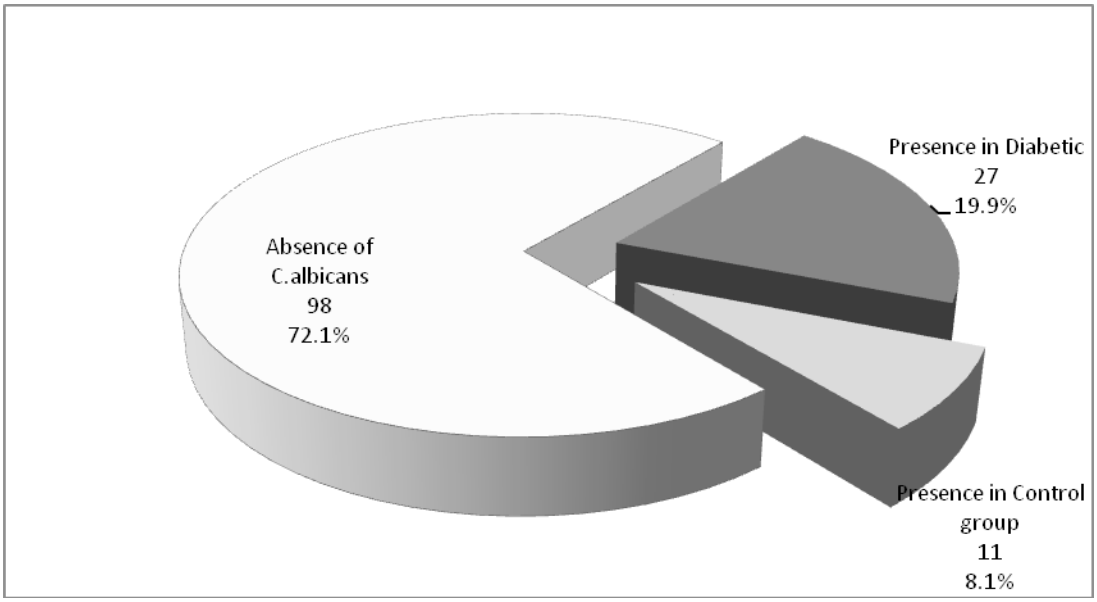

Figure 3. Relationship between *C. albicans*, periodontitis and diabetes diseases.

with diabetes and those without diabetes are presented in figure 1. The distribution of periodontal bacteria in cases of chronic periodontitis in diabetic compared with those with no diabetes are presented in figure 2. For chronic periodontitis, *E. corrodens*, *Campylobacter*, and *Eubacterium* were more noticeable in diabetic patients compared with those with no diabetes. Furthermore, cultures isolated *C. albicans* from 27 diabetic patients (19.9%) and from 11 control group patients (8.1%) with chronic periodontitis (Figure 3).

Discussion

Several workers have described diabetes as a risk factor for periodontal disease (19). Aggressive periodontal diseases are associated with diabetes (19-21), and there is evidence of an association between periodontal disease, especially severe periodontitis, and a variety of systemic conditions, among them other cardiovascular disease, including endocarditis and coronary heart disease, diabetes, and respiratory disease (21). The role of the immune system in the pathogenesis of diabetes and periodontal diseases is well documented (19). In our study, we found that chronic periodontitis was more frequently evident than aggressive periodontitis. We elucidated further the relationship between periodontitis, periodontal pathogens and diabetes. We found that the flora of aggressive periodontitis is richer and more widely varied flora than chronic periodontitis. In fact, we found no *Capnocytophaga, B. fragilis, P. anaerobius, P. micros, E. nodatum, K. pneumoniae, P. aeruginosa* and *S. aureus* in patients with chronic periodontitis. There was no difference between patients with diabetes and those without diabetes in the rates of isolation of periodontal pathogen bacteria in the cases of aggressive periodontitis. Previous studies from our region reported that periodontal pathogens were recovered in both diabetics in non-diabetics (22). Preshaw et al. (23) recovered several periodontal pathogens such as *Aggregatibacter actinomycetemcomitans*, *Campylobacter rectus, Capnocytophaga spp, Eikenella corrodens, Fusobacterium nucleatum* and *Prevotella intermedia*. These were similar in both diabetic and non-diabetic patients, but individuals with diabetes were more significantly affected by *P. gingivalis*. Similarly, the same group of workers reported, in a study of young Japanese individuals with type 1 diabetes mellitus, that a greater proportion of patients with periodontitis harboured *P. gingivalis* and *P. intermedia* than those who were considered periodontally healthy. It was suggested that in the case of diabetic patients, concentrations of oral microbial flora are increased due to higher concentrations of glucose in saliva and crevicular fluid (4). Other authors have shown diabetes as a risk factor which may favor the development of periodontitis and secondly, that periodontal infection seems to affect glycemic control (24). Therefore, cooperation between endocrinologists and dental surgeons is vital. Endocrinologists must be informed of the presence of periodontal pockets of infection in a given patient. Some studies reported that more diabetic patients had higher levels of periodontal pathogens than non-diabetic patients (22), but perhaps more likely that there is a two-way relationship between periodontal disease and glycemic control (24).

Several studies reported that the incidence of periodontitis is 2-3 fold higher in diabetic patients than in non-diabetic patients, and among males the presence of periodontal pathogens were positively associated with periodontitis (22). Coexistence between periodontitis and diabetes and the risk of periodontitis has been suggested to be 3-4 times in diabetic patients than in the general populations (6). Furthermore, in another series patients with diabetes had a three-fold higher risk for periodontal disease compared with non-diabetic patients after controlling for age, sex, and other confounding factors (19). Though the relationships between glycemic control and periodontal disease was considered weak (24). We identified some bacteria with higher rates in diabetic patients with aggressive periodontitis than in non-diabetic patients such as *P. anaerobius* (3.2 versus 0.5%). Similarly, *E. corrodens* (25.4%) and *Eubacterium sp* (8.5%) were isolated more often from diabetic patients with chronic periodontitis. This is in agreement with Abass and Omer (25) who found the same microorganisms in both groups, but there was differences in isolate numbers among the two groups. Mandell and co-workers suggested that diabetes may impair neutrophil adherence, chemotaxis, and phagocytosis, which may facilitate bacterial persistence in the periodontal pocket and significantly increase periodontal destruction (26).

Periodontitis affected the diabetic state, in which periodontal pathogens and cytokines probably increased insulin resistance by inhibiting glucose incorporation into the smooth muscle cells (27). Makiura *et al*. (27) also reported that *P. gingivalis* is shown as the microorganism most frequently detected in periodontal pockets of patients with type 2 diabetes and *A. actinomycetemcomitans* was not different in diabetics and non-diabetics. The frequency of *A. actinomycetemcomitans* was variable in the different periodontal sites in both groups (17). Early detection of *A. actinomycetemcomitans* in the oral cavity has been suggested to impact on health beyond the oral cavity (28).

Greater levels of *B. forsythus, A. actinomycetemcomitans* and *P. gingivalis* were found among diabetic patients with periodontitis (22). This is at variance with others who reported low rates of *A. actinomycetemcomitans* in the order of 5% only (29,30). In our own series, we found that *Capnocytophaga* was cultivated more in patients with diabetes (3.4%) than in the control group (2.2%) but only in patients with aggressive periodontitis. The numbers of *A. actinomycetemcomitans, Capnocytophaga spp*. and 'anaerobic vibrios' were high in samples of subgingival plaques in young diabetics. The presence of *Capnocytophaga* has been cited by many studies in diabetics vs non-diabetics (4) and specifically in patients with insulin-dependent diabetes (28). We identified *Campylobacter* in all patients with relatively similar percentages. Bacteria of periodontal pockets, such as *C. rectus* and other microorganisms can cause extra-oral infections (31). We found that the rate of *P. intermedia* was low in diabetic patients with chronic periodontitis compared to controls while *E. corrodens* was more often in diabetics suffering of chronic periodontitis. Diabetics in general were described to have greater levels of *P. intermedia, P. melaninogenica, Bacteroides gracilis, Eikenella corrodens, Fusobacterium nucleatum and Campylobacter rectus* than normal people. In another study, *A. actinomycetemcomitans*, *P. gingivalis* and particularly *P. intermedia* are high in the sites from subjects with diabetes with periodontitis (32). Kim *et al*. (33), found that *E. corrodens*, *T. denticola* and *P. gingivalis* may play an important role in periodontitis patients with both type 1 and type 2 diabetes.

In our study, the rate of *Staph. aureus* was almost identical in diabetic and non-diabetics with aggressive periodontitis and almost absent in patients with chronic periodontitis. *Staphylococcus epidermidis* has been reported in diabetics compared to non-diabetics (4). Smith *et al*. (34) reported that *Staph. aureus* isolated from the sub-gingival plaques of diabetic patients. Abass and Omer (25), found *Staph. aureus* in 15.5% in diabetics patients contrasted with only 4% in control cases. They showed that total number of isolates was generally more among diabetics with the exception of *Staph. epidermidis* which was higher in controls. We identified *P. aeruginosa* and *E. coli* in 3.2% of our diabetic patients. This is at variance with Abass and Omer (25) who isolated *E. coli* in 14.5% cases with diabetes and only 4% in the control group. We isolated *Strep. viridans* in 40.3% of diabetic patients with chronic periodontis and only in 8.7% in diabetics patients with aggressive periodontitis. Abass and Omer (25), found *Strep. viridans* in 43% of diabetics and 24% in controls. We identified *C. albicans* in 19.9% of diabetic patients with periodontitis compared to the controls (8.1%). Kim *et al*. (34), reported that *C. albicans*, can play an important role in periodontitis in patients with both types of diabetes. Abass and Omer (25), isolated *C. albicans* in 37.5% of diabetic patients and 4% in the control group. Sardia *et al*. (17), reported that 85% of their patients were colonised by *Candida* species in one or more sites evaluated. In the diabetic group, all tested *Candida* spp. (*C. albicans*, *C. dubliniensis*, *C. tropicalis* and *C. glabrata)* were found in periodontal pockets while in the non-diabetic group, *C. albicans* and *C. dubliniensis* were most prevalent. Kumar *et al*. (35), found that *Candida* was diagnosed in 83.6% of type 1 and 68.5% of type 2 diabetes and only in 27% of those with no diabetes.

We conclude that periodontal pathogens are distinctly different in patients with diabetes from those without diabetes. The differences are evident in both aggressive and chronic periodontitis. Furthermore, the flora of diabetic patients with aggressive periodontitis was characterized by a rich flora and varied especially amongst the type of Gram negative rods. It is noteworthy, that the presence of *A. israelii* was evident in diabetics only and *P. anaerobius* and *E.coli* were present in this group compared to the control. *C. albicans* was predictably associated with hyperglycemia in our patients. These different in microbiological characteristics are relevant in both dental and general medical practices.

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