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Original Article

MICROBIOLOGICAL STUDY OF ORAL FLORA IN DIABETIC PATIENTS WITH GINGIVITIS

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ABSTRACT

Objective: Given the importance of the association between diabetes and periodontal disease, the main objective of the present study was to compare the microbial diversity responsible for gingivitis in patients with and without type 2 diabetes.

Methods: Samples were collected from the oral cavity of 134 patients with gingivitis and categorised into 3 groups (68 non-diabetic patients and 66 diabetic patients; 33 with controlled diabetes and 33 with poorly controlled diabetes). Sample culture was carried out on selective culture media. The identification of isolated strains involved a series of biochemical tests including miniature galleries (API 20E and 20 Strep), the traditional biochemical gallery (tubes) and automated bacterial identification (BD Phoenix™).

Results: Identification by biochemical methods made it possible to differentiate 14 bacterial species and one yeast. There was greater bacterial diversity in diabetic patients as compared to non-diabetic patients. Periodontal pathogens were isolated from both diabetic and non-diabetic populations; however, certain microbes such as *Streptococcus acidominimus, Enterobacter cloacae, Klebsiella oxytoca,* and *Pseudomonas aeruginosa* were present only in diabetics, with a much higher percentage in those with poorly controlled diabetes.

Conclusion: Poorly controlled diabetes causes metabolic dysregulation that can increase the severity of periodontal disease.

Keywords: Diabetes, Periodontal disease, Glycemic balance, Microbial diversity

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INTRODUCTION

Oral diseases in diabetic patients have accelerated progression when the control of blood glucose is inadequate [1]. Advanced hyperglycemia may cause impairment of salivary gland function, the proliferation of pathogenic microorganisms, and progression of gum disease [2-4].

The prevalence of periodontal disease in diabetics is highly variable. It has been shown that 100% of diabetic patients had periodontal disease compared with 50% of control individuals [4, 5].

It is well documented that diabetics are more likely to develop periodontal infections than individuals without diabetes [6, 7]; with a reported prevalence of 9.8% in type 1 diabetics compared with 1.6% in non-diabetic patients. In patients with type 2 diabetes, which is the most common form of diabetes (95% of the diabetic population [8]), the risk of periodontal disease is three times higher than in the general population [9].

It is not well known that periodontal disease is considered the sixth greatest complication of diabetes, especially when the disease is not appropriately controlled [10, 11], and determines changes in the bacterial population and production of inflammatory mediators, which reduces the effectiveness of the host response [12]. Controlled diabetes does not pose a major risk of periodontitis, and the results of initial and surgical periodontal therapy are better in these patients. In addition, periodontal therapy can reduce insulin demand in diabetics [13].

As a result, periodontal diseases and diabetes have a bi-directional link [14]. The treatment of diabetes therefore also involves the treatment of periodontal disease and the maintenance of rigorous oral hygiene [15]. Reciprocally, healthy periodontal status and good plaque control will, in turn, improve glycemic control, resulting in a significant reduction in glycated hemoglobin (HbA1c) [16].

Given the importance of the association between diabetes and periodontal disease, the main goal of the present study was to investigate how diabetes affects host-bacterial interactions by focusing on the microbial diversity responsible for gingivitis in patients with and without type 2 diabetes.

MATERIALS AND METHODS

The present study was carried out at the Dental Office of the Centre for Chronic Diseases, Sale, Morocco. Before taking the samples, all participants signed their consent.

Target population

-Group I: 68 non-diabetic subjects.

-Group II: 33 diabetic subjects with controlled diabetes.

-Group III: 33 diabetic subjects with poorly controlled diabetes.

Sample collection

Swab specimens were taken from the oral cavity of diabetic and non-diabetic patients with gingivitis and streaked onto culture media. Subjects who used antiseptic mouthwashes during the week prior to collection, as well as those who took antibiotics in the previous month, were excluded from the study. Individuals of both genders were included, who did not suffer from systemic diseases other than type 2 diabetes, were non-smokers, and did not carry dentures or orthodontic appliances. Pregnant or breastfeeding women were also excluded from the present study.

Seeding and isolation

Sample seeding was carried out on cystine lactose electrolyte deficient (CLED) or bromocresol purple (BCP) medium and incubated at 37°C for 24 h. Purification of bacterial colonies was performed by successive subcultures on the same medium or another selective medium.

Pseudomonas aeruginosa strains were isolated on nutrient agar supplemented with cetrimide, *Escherichia coli* and *Klebsiella* strains

were isolated on methylene blue eosin (MBE) agar, and *Streptococcus* and *Enterococcus* strains were isolated on blood agar. All plates were incubated at 37 °C in a 10% CO_2 atmosphere for 24 h [17].

Identification of isolated pathogens

Identification of strains was based on a series of preliminary tests (appearance of colonies, Gram stain), biochemical tests (catalase, oxidase, coagulase, and DNase), and identification by miniature galleries (API 20E and 20 Strep), the traditional biochemical gallery (tubes), and automated bacterial identification (BD Phoenix™) [18, 19].

Statistical analysis

The data were analyzed using SPSS (Statistical Package for Social Sciences) software version 21. The Kolmogorov-Smirnov test was used to study the distribution of the quantitative variable, age, which is expressed as the median and quartile. Comparison of the qualitative variables among the three populations (gender, hygiene parameters, and microbial flora) was carried out by the Pearson khi2 test or Fisher's lowest difference test (LSD). The Mann Whitney test was used to compare variables with non-Gaussian distribution (age of patients). A value of $p<0.05$ is considered statistically significant.

RESULTS

Distribution of the studied population

Statistical analysis allowed us to divide our samples according to the demographic and hygiene parameters presented in table 1.

Table 1: Distribution of the diabetic and non-diabetic populations by demographic and hygiene parameters

Variables	Study population n=134		*p*
	Non-diabetic patients n = 68	Diabetic patients n = 66	
Age[a]	55 (39.75-60)	50 (33.50-59.25)	0.168
Gender[b]			0.491
Female	44(31.4%)	48(68.6%)	
Male	24(40.0%)	18(60.0%)	
Calculus[b]			0.650
Absent	18(30.0%)	21(70.0%)	
Present	50(35.7%)	45(64.30%)	
Brushing[b]			0.827
No	26(36.1%)	23(63.9%)	
Yes	42(32.8%)	43(67.2%)	

[a]Values are expressed as the median and quartiles, [b]values are expressed as numbers and percentages. $p<0.05$ is considered statistically significant. The median age of the population was 52 (36-60) with extremes of 21 and 66 y old. The distribution by gender revealed a female predominance of 70% and a gender ratio of 0.42. The oral examination revealed the presence of calculus in 70% of the study population. 63% of the entire population brushed their teeth daily.

Identification of isolated pathogens

15 strains were isolated, and their identification revealed 14 bacteria including 8 genres; *Streptococcus, Enterococcus, Staphylococcus, Enterobacter, Escherichia, Klebsiella, Pseudomonas,* and *Lactobacillus*, as well as one yeast *Candida albicans* (table 2).

Periodontal pathogens were present in both diabetic and non-diabetic populations. There was greater bacterial diversity in diabetic patients compared with non-diabetic subjects, with certain pathogens such as *Streptococcus acidominimus, Enterobacter cloacae, Klebsiella oxytoca,* and *Pseudomonas aeruginosa* only being present in diabetics.

Table 2: Distribution of pathogens isolated from the oral flora of diabetic and non-diabetic patients with gingivitis

Strain	Study population =134		*p*
	Non-diabetic patients n = 68	Diabetic patients n = 66	
Streptococcus constellatus	4(18.2%)	9(81.8%)	0.324
Streptococcus acidominimus	0(0%)	16(100%)	0.002
Streptococcus oralis	14(26.9%)	19(73.1%)	0.373
Enterococcus faecalis	10(26.3%)	14(73.7%)	0.432
Streptococcus spp	14(58.3%)	5(41.7%)	0.1
Streptococcus sanguinis	10(50%)	5(50%)	0.301
Streptococcus mitis	12(50%)	6(50%)	0.329
Streptococcus mutans	2(100%)	0(0%)	0.113
Staphylococcus aureus	6(30%)	7(70%)	1.000
Enterobacter cloacae	0(0%)	20(100%)	0.001
Klebsiella oxytoca	0(0%)	14(100%)	0.002
Escherichia coli	2(12.5%)	7(87.5%)	0.259
Pseudomonas aeruginosa	0(0%)	7(100%)	0.002
Lactobacillus acidophilus	6(30%)	7(70%)	1.000
Candida albicans	8(28.6%)	10(71.4%)	0.767

Values are expressed as numbers and percentages. $p<0.05$ is considered statistically significant.

The distribution of the diabetic population according to glycemic balance revealed that 50% of the population presents with poorly controlled diabetes (fig. 1).

It is noteworthy that the percentage of ten microbial species identified in diabetic patients with poorly controlled diabetes is much higher than that in the group with controlled diabetes (*Streptococcus constellatus* (48%), *Streptococcus acidominimus* (63%), *Streptococcus oralis* (43%), *Enterococcus faecalis* (53%), *Staphylococcus aureus* (60%), *Enterobacter cloacae* (80%), *Klebsiella oxytoca* (93%), *Escherichia coli* (63%), *Pseudomonas aeruginosa* (100%), and *Candida albicans (58%)*. Moreover, these species are also present in very high numbers as compared with non-diabetic subjects.

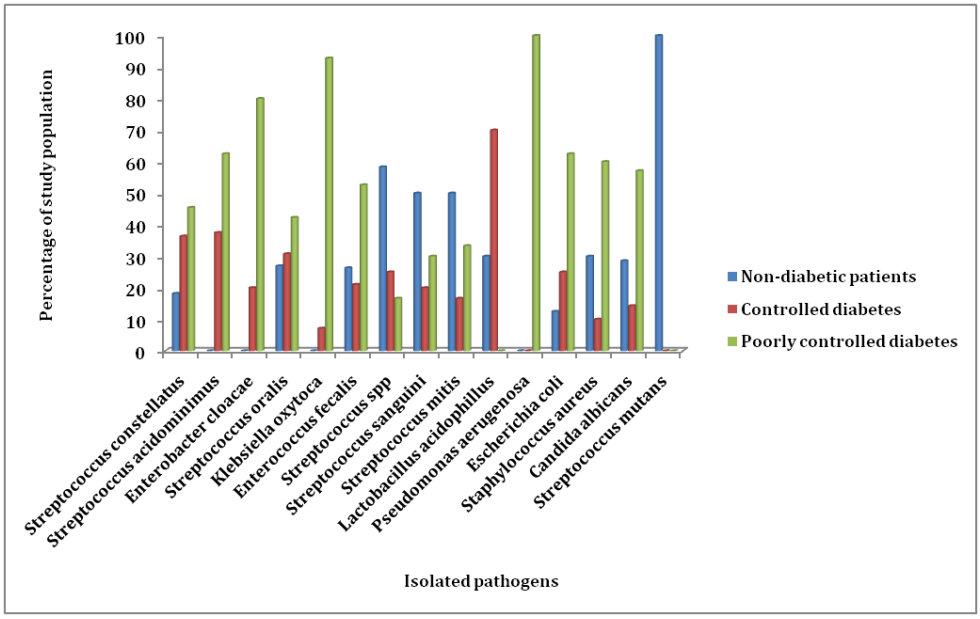

Fig. 1: Distribution of microbial species according to glycemic equilibrium within the study population

DISCUSSION

Diabetes can cause many serious systemic complications, with the classics including retinopathy, nephropathy, neuropathy, macrovascular, cerebrovascular, cardiovascular, and peripheral disorders [20], in addition to major alterations in the healing process. Increasing scientific evidence has also linked diabetes with periodontal disease [21, 22]. The objective of the present study was to compare the microbial diversity responsible for gingivitis between diabetic and non-diabetic patients.

Based on clinical and radiological parameters, diabetic patients have shown an aggressive tendency towards bone loss as compared with healthy controls, suggesting that diabetes influences periodontal tissue destruction [23]. This is in accordance with studies that have demonstrated that a subject with type 2 diabetes is 2.8-3.4 times more likely to develop periodontal disease than a healthy subject [23, 24]. Numerous epidemiological studies have shown that the incidence and prevalence of periodontitis, in addition to the severity of periodontal destruction, are higher in patients with type 1 and type 2 diabetes [25-29]. Nelson *et al.*, have shown that the prevalence of periodontal disease is approximately 60% higher in diabetic patients as compared with their non-diabetic counterparts [30].

In the present study, the bacteriological analysis showed a high concentration of microbial species considered as pathogenic (*Streptococcus acidominimus, Enterobacter cloacae, Klebsiella oxytoca,* and *Pseudomonas aeruginosa)* in diabetic subjects as compared with non-diabetic subjects, which is consistent with results reported by Sbordon *et al.*, [31]. In patients with poorly controlled diabetes and periodontal problems, the percentage of the most virulent microbial species was higher than in subjects with controlled diabetes and non-diabetic subjects, which is in agreement with data reported by Seppaa and Ainamo [32]. Epidemiological studies have also shown that type 2 diabetes can be predictive of periodontal disease when the systemic conditions are poorly controlled [33, 34], in addition to responding poorer to surgical and non-surgical periodontal treatments [35, 36].

CONCLUSION

The results of the present study suggest that poorly controlled diabetes causes metabolic dysregulation that can increase the severity of periodontal disease. Numerous studies have shown that diabetes is associated with an increased risk of periodontal disease, which can be considered the sixth true complication of diabetes. Similarly, an oral infection can influence blood glucose control, insulin resistance, and the occurrence of complications associated with diabetes. Although more powerful studies are needed to complete the demonstration of the link between periodontal disease and glycemic control, we can already recommend integrating rigorous oral examination and necessary periodontal care (preventive and curative) into the management of diabetic patients.

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AUTHORS CONTRIBUTIONS

The first author has carried out the research. Second third and fourth authors have provided study conception, the design of work, drafting of the manuscript and critical revision.

CONFLICT OF INTERESTS

Declared none

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