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Metabolic control, oral microbiological and periodontal conditions in patients with diabetes mellitus

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Aim: To investigate the relationships between metabolic control, duration of disease, type of diabetes, diabetes-related complications, and microbiological and clinical periodontal conditions in subjects who have diabetes mellitus.
Study design: A cross-sectional analysis of clinical and microbiological periodontal conditions was performed in 41 subjects with diabetes mellitus, untreated for any form of periodontal disease.
Results: Twenty-seven subjects with type 1 diabetes mellitus (T1DM) and 14 with type 2 (T2DM) (mean duration, 17.4 years [SD 9.3], and 11.7 years [SD 4.2], respectively) were studied. The mean serum values of glycosylated haemoglobin (HbA1c) did not differ between the two groups (T1DM, 7.7% [SD 1.8]; T2DM, 8.0% [SD 0.9]). Medical complications were present in 45% of the T1DM and in 71% of the T2DM subjects. Gingivitis was not correlated with mean HbA1c levels in T2DM subjects, but was negatively correlated with the T1DM group ($r^2 = 0.19$, Pearson's coefficient $r = - 0.43$, $p < 0.02$). The presence of bacteria in periodontal pockets differed with T1DM and T2DM status for *Campylobacter ochracea*, *Peptostreptococcus micros*, *Porphyromonas gingivalis* and *Selenomonas noxia*. Oral bacterial load was not linked to serum HbA1c levels or the presence of diabetes-related complications.
Conclusions: Gingivitis was common and similar in both T1DM and T2DM subjects. Serum HbA1c levels were not related to the levels of the subgingival microbiota in T2DM, but negatively correlated with the extent of gingivitis in T1DM. *P. gingivalis* appeared to be more prevalent in T2DM subjects.

■ Introduction

Diabetes mellitus (DM) is a disease with increasing prevalence. The World Health Organization (WHO) projects that diabetes-related deaths will increase by more than 50% in the next 10 years. Pathological characteristics of DM such as duration of the disease[1] and the level of metabolic control (glycosylated haemoglobin levels [HbA1c])[2] have been associated with the prevalence of periodontitis. While it remains unclear if treatment of periodontitis could significantly reduce serum HbA1c levels in subjects

with DM[3], there are reasons to believe that periodontal inflammation might influence the status of diabetes and plasma glucose levels[4,5].

The role of the infectious aetiology of periodontitis and its implications for DM remain poorly understood. Existing studies have mainly investigated the prevalence of periodontal pathogens in populations of either type 1 diabetes mellitus (T1DM) or type 2 (T2DM) patients. Available data suggest that *Porphyromonas gingivalis*, *Treponema denticola*, *Eikenella corrodens* and *Candida albicans* may play important roles in both T1DM and T2DM[6]. In subjects with poorly controlled DM, a significantly higher percentage of *Prevotella intermedia* has also been found at sites exhibiting deep pockets and attachment loss[7]. In contrast, studies of subgingival plaque samples examined by immunofluorescence methods have detected a high prevalence of black-pigmented *Bacteroides* and *P. gingivalis,* but not *P. intermedia* in T2DM subjects[8]. Thus, different studies have identified that high concentrations of *P. gingivalis*[1,9], *Tannerella forsythensis*[1,9], *P. intermedia*[10] and *Capnocytophaga* species[11] can be identified in subjects both with T1DM or T2DM and periodontitis. The presence of *P. gingivalis* has also been shown to be related to a compromised response to a periodontal treatment in T1DM[12].

A link between insulin-dependent diabetes mellitus (IDDM), periodontitis and cardiovascular disease has also been identified where *P. gingivalis* presence in T2DM subjects has been associated with carotid atherosclerosis[13]. Whether differences in the subgingival microbiota exist between T1DM and T2DM patients remains largely unknown. The relationship between HbA1c levels and subgingival microbiota is also unknown.

The aims of the present study were to (I) investigate the relationships between level of metabolic control, duration of disease, type of diabetes, presence of diabetes-related complications, and the microbiological and clinical conditions of the periodontal tissues in T1DM and T2DM subjects, and (II) to study possible differences in the infection patterns between T1DM and T2DM patients.

Consequently, the null hypotheses of the study were (I) there is no correlation between metabolic control, duration of disease, type of diabetes and presence of diabetes-related complications with the microbiological and clinical conditions of the periodontal tissues in T1DM and T2DM subjects and (II) there is no difference in the qualitative or quantitative bacterial infection pattern between T1DM and T2DM patients.

■ Study design

The present study was approved by the local ethics committee (Kantonale Ethikkommission, KEK, Bern, Switzerland). All registered diabetic patients in the year 2003 at the Department of Endocrinology and Diabetology of the University Hospital at the University of Berne, Switzerland, were invited to participate in the present study. The inclusion criteria were: age between 18 and 70, confirmed diagnosis of diabetes mellitus (T1DM or T2DM), minimum duration of diabetes of at least 2 years, complete documentation and registration of endocrine conditions, compliance with study regulations and informed consent. The exclusion criteria were: current or ex-smokers (absence of smoking for less than 5 years), edentulous patients, any form of treatment for periodontal disease during the last 6 months, use of antibiotics in the last 6 months, the use of medication known to affect periodontal tissues and pregnancy.

All patients were clinically examined by one examiner (MK), thus eliminating the risk of intra-examiner variation. The examiner was blinded to patients' type, duration, level of metabolic control of diabetes and presence of any complications. Clinical examination included measurements of probing pocket depth (PD), clinical attachment level (CAL), bleeding on probing (BOP) and dichotomous plaque index (PI). Patients diagnosed with periodontitis were invited to receive treatment at the Department of Periodontology and Fixed Prosthodontics, School of Dental Medicine, University of Berne.

A total of 4 subgingival bacterial samples were taken from each patient. The samples were collected with a sterile curette from the periodontal pocket of the deepest site in each quadrant. Microbiological samples were pooled and analysed with the DNA-DNA hybridisation technique, measuring a bacterial load of 40 known pathogens[14]. Subject mean values for the different species were calculated to represent the mirobiota of the subject.

The endocrine data were collected through the patients' medical records. The following information was provided for each patient: endocrine diagnosis, duration of disease, level of metabolic control (HbA1c level) for the year 2003 (mean of 4 values) and presence of diabetes-related complications in the year 2003 (retinopathy, polyneuropathy, nephropathy/microalbinuria, dislipidaemia and other complications such as erectile dysfunction, peritonitis, and vascular/cardiovascular disorders).

■ Statistical analysis

Statistical analysis included descriptive and comparative methods. Independent *t* test, not assuming equal variance, was used to assess differences in microbial counts by diabetes status. Correlations between parameters were assessed by r^2 and Pearson's coefficient. Receiver operator characteristic (ROC) curve analysis was additionally used to identify bacteria that discriminated diabetic status.

■ Results

Forty-one patients met the inclusion criteria and agreed to participate in the study. Twenty-seven patients were diagnosed with T1DM and 14 with T2DM. Subjects in the T1DM group (mean 41.4 years [SD 13.3]) were significantly younger than those in the T2DM group (56.0 years [SD 13.8], 95% CI 5.7–23.5 years, $p < 0.001$). The duration of DM also differed in that T2DM subjects had been diagnosed with DM for an average for 17.4 years (SD 9.3), and the T2DM subjects had been diagnosed with DM for an average of 11.7 years (SD 4.2, 95% CI 0.4–11.1, $p < 0.01$). The majority of patients presented similar values of HbA1c in all 4 samplings and only 2 patients (5%) had a difference > 1 between the highest and lowest value in the year. The mean serum HbA1c values in 2003 did not differ significantly between the two groups (T1DM 7.7% [SD 1.8], T2DM 8.0% [SD 0.9]). In the T1DM group, 64.6% of subjects had an average HbA1c level > 6.5%, while in the T2DM group 100% had an HbA1c level above this threshold value.

Forty-five per cent of the T1DM subjects and 71% of the T2DM subjects had diabetes-related medical complications. In the T1DM group, the most common complications were retinopathy (18.5%), microalbuminuria (18.5%) and neuropathy (11.1%). The most common complications in the T2DM group were neuropathy (42%), microalbuminuria (21.4%) and retinopathy (18%).

■ Dental and diabetic status

The number of remaining teeth differed significantly by DM status. T1DM subjects had, on average, 26.0 teeth (SD 5.3), while T2DM subjects had an average of 21.1 teeth (SD 8.0, 95% CI 0.7–9.1, $p < 0.02$). Oral hygiene was poor in both groups, with an average of 60.7% (SD 19.8) of tooth sites containing visible plaque in the T1DM group, and 62.6% (SD 23.9, $p = 0.12$) of sites containing visible plaque in the T2DM group. The extent of gingival inflammation, as defined by gingival units bleeding on probing, was 61% in both groups. The BOP score was not correlated with the mean HbA1c levels for the T2DM group. The BOP score was, however, negatively correlated with the mean HbA1c levels for the T1DM group ($r^2 = 0.19$, Pearson's coefficient $r = -0.43$, $p < 0.02$).

Six subjects with T1DM (22%) and 7 subjects with T2DM (50%) had 4 or more sites with PD > 5 mm and were declared as having periodontitis. Clinical attachment levels differed in that subjects in the T2DM group showed significantly greater evidence of CAL loss (mean loss 0.9 mm, SD 1.9) versus the T1DM group (mean loss 0.2 mm, SD 0.3, 95% CI 0.3–1.2 mm, $p < 0.02$).

In the T1DM group, the serum mean HbA1c level was significantly correlated to the mean CAL level ($r^2 = 0.19$, Pearson's coefficient $r = 0.47$, $p < 0.05$). In the T2DM group, the serum mean HbA1c level was not significantly correlated with the mean CAL level ($r^2 = 0.01$, Pearson's coefficient $r = 0.12$, $p < 0.66$). There was no relationship between serum mean HbA1c values and the extent of sites with PD at > 5 mm in either group.

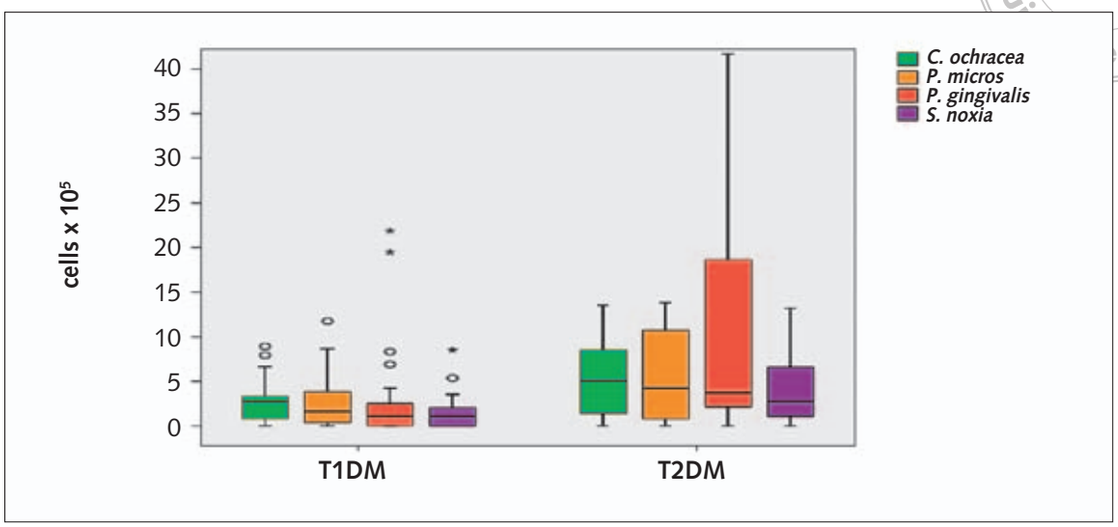

Fig 1 Box plot diagram presenting levels of *Capnocytophaga ochracea, Peptostreptococcus micros, Porphyromonas gingivalis* and *Selonomonas noxia* in subgingival samples of T1DM and T2DM subjects.

BOP scores, supragingival plaque scores and PD in subjects with T1DM and T2DM in relation to the sub-gingival microbiota

In subjects with T1DM, only the level of *P. intermedia* was correlated to the percentage with BOP (r^2 = 0.25, Pearson's coefficient r = 0.50, p< 0.01). No significant correlations were found between the 40 microbial species tested and per cent of BOP in subjects with T2DM. In subjects with T1DM only the level of *P. intermedia* was correlated with the plaque score (r^2 = 0.28, Pearson's coefficient r = 0.53, p < 0.01). No statistically significant correlations were found between the 40 microbial species tested and the percentage of surfaces visible with supra-gingival plaque in T2DM subjects. No statistically significant correlations between the proportion of sites with PD = 4, 5 or > 6 mm and subgingival bacterial loads were found in the T1DM and T2DM groups.

Subgingival microbiota in subjects with diabetes mellitus (T1DM and T2DM)

The distribution of subjects who had a mean value of bacteria present in the subgingival samples larger than 1 x 10^4 or 1 x 10^5 were analysed. Thus, 51.9% of the subjects in the T1DM group and 78.4% of the subjects in the T2DM were positive for *P. gingivalis*. Data analysis identified statistically significant differences by endocrine status for several species. Independent *t* tests (equal variance not assumed) indicated significantly higher bacterial loads for 4 species (*Capnocytophaga ochracea*, *Peptostreptococcus micros*, *P. gingivalis*, and *Selenomonas noxia*, Fig 1) in subjects with T2DM. No correlations were found between the duration of diabetes and the subgingival bacterial load of individual bacteria or for the total bacterial load.

In the T1DM group, the serum mean HbA1c levels were statistically significantly negatively correlated with the levels of *P. intermedia* (r^2 = 0.23, Pearson's coefficient r = - 0.48, p < 0.01). No correlations were found between bacterial load for any of the 40 species studied and HbA1c levels in the T2DM group. When the proportion of sites with a PD > 5.0 mm was calculated, significant correlations were found with bacterial loads for *P. gingivalis* in the T2DM group (r^2 = 0.22, Pearson's coefficient r = 0.59, p < 0.05). No such correlations were found in the T1DM group.

Analysis by ROC identified that two bacterial species, *P. gingivalis* and *S. noxia*, discriminated between T1DM and T2DM (Table 1).

Discussion

The present study population had received regular medical attention at the Department of Endocrinology and Diabetology of the University Hospital in Berne. The mean serum HbA1c levels suggested that a majority of subjects were in the higher range of values. The plasma glucose level as expressed by the

	Area	SE	Asymptotic Significance	Asymptotic 95% Confidence Interval	
Test result variable(s)				Lower bound	Upper bound
P. gingivalis	0.737	0.087	0.014	0.566	0.907
S. noxia	0.730	0.084	0.017	0.565	0.895

Under the nonparametric assumption
Null hypothesis: true area = 0.5

Table 1 Assessent of the area under the curve in ROC curve analysis identifying *P. gingivalis* and *S. noxia* as being the only bacteria among the 40 species studied that discriminated between T1DM and T2DM.

HbA1c score can vary significantly with time. The value used in the present study is the mean of a minimum of 4 samples, taken on a quarterly basis. A risk still exists that a mean value might mask wider variations throughout a year, however, as these patients were under regular medical attention and control, extreme variations were not expected. By contrast, HbA1c values for the great majority of patients appeared rather similar in all 4 samples. The ratio between the number of subjects having T1DM and T2DM in the sample of this study reflects the characteristics of the specialised hospital clinic and is therefore not the ratio expected in the general population.

The results of this study can serve as pilot observations in a field where little is known, yet general interpretations should be made with caution due to certain limitations. In particular, one should note that the sample represents the composition of the selected population registered in a tertiary endocrinology clinic and does not reflect the normal distribution of DM subjects. An age-matched control group was not possible due to sample limitations, which does not allow eradication of age as a confounding factor. The large number of parameters studied, in particular the number of bacterial species, suggests that some species could have shown correlations due to statistical chance alone. Some of the conclusions reached in the present study are in agreement with previous research; others need to be confirmed by larger scale future studies.

The periodontal data demonstrated that extensive gingival inflammation and periodontitis was commonly diagnosed in both groups. It is of interest that among periodontal indices, only the CAL differed significantly between the T1DM and T2DM groups, with more evidence of attachment loss in T2DM. This might be explained by the fact that the T2DM subjects were older than T1DM subjects. Issues of smoking as a contributing risk factor for periodontitis were eliminated in the present study, as only subjects without a history of a smoking habit were included.

The severity of gingival inflammation, the poor oral hygiene and prevalence of periodontitis in both groups may partly be explained by the fact that these subjects had not received any form of periodontal treatment, at least not during the last 6 months. The T2DM subjects had fewer teeth that the T1DM subjects, which might be explained by the fact that the T2DM subjects were older. On the other hand, T1DM subjects had a longer history of being diagnosed with diabetes, suggesting that diabetes is not clearly related to tooth loss.

It is, however, also possible that T2DM subjects had undetected diabetes for several years, which made them susceptible to oral bacterial infections and resulted in the need for tooth extractions. Other studies suggest that tooth loss is similar between T1DM and T2DM in older subjects[15]. T2DM subjects presented with many more diabetes-related complications than the T1DM subjects. This again may be partly explained by age differences. Yet the presence of complications was not related to periodontal or microbiological status for both groups.

Available literature suggests a relationship between low-grade inflammation and serum HbA1c values[16]. A relationship between the extent of CAL in young T1DM subjects and serum HbA1c levels has been reported[17]. Experimental gingivitis studies of subjects with T1DM have suggested a relationship between the presence of key pathogens in periodontal pockets and the severity of gingivitis[18].

Although periodontal conditions may improve in subjects with poorly controlled diabetes as a result of initial cause-related periodontal therapy, the impact on serum HbA1c levels remains questionable[3]. Even in cases where a reduction in serum HbA1c occurs, it is difficult to evaluate the impact of such a reduction on the diabetic conditions. Short-term reductions in subgingival bacterial levels have been reported[18]. Subgingival instrumentation may, however, only have temporary effects and might rather result in increasing levels of such bacteria, as has been demonstrated in subjects without a history of diabetes mellitus[19]. Periodontal therapies may in the short term mask host inflammatory responses. Follow-up periodontal intervention studies of subjects with T1DM and T2DM with a focus on the microbiota is needed.

The predisposition to bacterial infections and chronic inflammation in diabetes may in part be related to the effects of hyperglycemia or other functional abnormalities, such as on polymorphonuclear leukocytes (PMN). The increased oxidative respiratory burst activity of PMN cells in diabetic subjects may predispose them to infection and chronic inflammation[20]. Priming of PMN cells in T2DM subjects can cause oxidative stress and self-necrosis[21]. This may partly explain the high level of gingival inflammation and the impaired ability of the cell-mediated immune system to eliminate subgingival bacteria in both types of DM, as shown in the present study. Others have shown that short-term studies of periodontal intervention in T1DM subjects failed to demonstrate changes in immunoglobulin G (IgG) titres in a subset of bacteria, or the ability to improve dental conditions or eliminate bacteria such as *T. forsythensis* and *P. gingivalis* from these subjects[9].

Independent *t* tests showed that 4 different species discriminated between T1DM and T2DM, but the ROC curve analysis confirmed this for only two of them (*P. gingivalis* and *S. noxia*). The ROC analysis confirmation was judged as necessary since, due to the large number of species controlled, a certain portion of correlations could be attributed to statistical chance only. The information on *S. noxia* and the role of the microorganism in periodontal disease is limited. It is known that *S. noxia* coaggregates with *Fusobacterium nucleatum*[22]. A majority of both T1DM and T2DM subjects carried *F. nucleatum* in their samples. There are no studies that specifically associate *S. noxia* with diabetes mellitus. In contrast, several studies have identified *P. gingivalis* as a key pathogen in periodontitis and also that this microorganism may play an important pathogenic role in subjects with T1DM[10,23,24]. In subjects with T1DM, detection of elevated serum glutamic acid decarboxylase (GAD) autoantibody levels in combination with elevated IgG titres to *P. gingivalis* has been shown to be indicative of an impaired response to periodontal therapy[25]. Although levels of GAD antibodies were not assessed in the present study, such observations also point to the difficulties in periodontal therapies of subjects with T1DM. This may be of importance, as the present study demonstrated that a very high proportion of T1DM and T2DM subjects were infected with a large number of pathogens, exceeding the threshold of what has been reported for non-diabetic subjects elsewhere[18,26]. The present data showing that *P. gingivalis* is commonly found in diabetes is in agreement with others[1,13,17].

In conclusion, both oral hygiene status and serum HbA1c levels in the study population were not sufficiently controlled. Various diabetes-related complications were common and similar in both T1DM and T2DM. This was also true for gingivitis and periodontitis. The presence of diabetes-related complications was however not correlated with the extent and severity of periodontal disease. The composition of subgingival microbiota suggested a severe infectious burden. The mean serum HbA1c level was not correlated with subgingival microbiota for T2DM subjects. However, the level of subgingival microbiota was negatively correlated with the extent of gingival inflammation for T1DM subjects. *P. gingivalis* appears to be more prevalent in T2DM subjects.

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