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Clinical and microbial oral health status in children and adolescents with type 1 diabetes mellitus†

Anastasia Babatzia[1], William Papaioannou[2], Anastasia Stavropoulou[3], Nikolaos Pandis[4,5], Christina Kanaka-Gantenbein[6], Liza Papagiannoulis[1] and Sotiria Gizani[1]

[1]*Department of Paediatric Dentistry, Faculty of Dentistry, National and Kapodistrian University of Athens, Athens, Greece;* [2]*Department of Preventive and Community Dentistry, Faculty of Dentistry, National and Kapodistrian University of Athens, Athens, Greece;* [3]*Virology Unit, Department of Microbiology, Medical School, National and Kapodistrian University of Athens, Athens, Greece;* [4]*Department of Orthodontics and Dentofacial Orthopedics, School of Dental Medicine/Medical Faculty, University of Bern, Bern, Switzerland;* [5]*Private Practice, Corfu, Greece;* [6]*Division of Endocrinology, Diabetes and Metabolism, First Department of Pediatrics, Aghia Sophia Children's Hospital, National and Kapodistrian University of Athens, Athens, Greece.*

Objectives: To study the oral health of young individuals with controlled and uncontrolled type 1 diabetes mellitus (T1DM) and compare the results with those for healthy counterparts. **Materials and Methods:** One-hundred and forty-four youngsters (6–15 years of age) were assigned, according to glycaemic control, to three study groups: (i) diabetic patients with poor glycaemic control [glycated haemoglobin (HbA1c ≥7.5%)] (n = 35); (ii) diabetic patients with good glycaemic control (HbA1c <7.5%) (n = 39); and (iii) healthy individuals (n = 70). Plaque, gingival inflammation, calculus and decayed, missing and filled surfaces (DMFS) indices were recorded. Salivary parameters were determined, and stimulated saliva was collected to allow detection and determination of the levels of oral *Candida albicans* and *Streptococcus mutans* by real-time polymerase chain reaction (PCR). **Results:** Significantly different amounts of plaque were found among the study groups (P = 0.024): youngsters with poor glycaemic control had significantly more plaque than youngsters in the other two groups. The gingival, calculus and DMFS indices were not significantly different among groups (P > 0.05). *Candida albicans* levels were not statistically significant different among groups, but the group with poor glycemic control showed an elevated frequency of detection. *Streptococcus mutans* was isolated from the oral cavity of 96 of the 144 individuals. A statistically significant difference in the level of *S. mutans* was found between the group with poor glycaemic control and the healthy control group (P = 0.032). **Conclusions:** The results imply that youngsters with T1DM have a lower level of oral hygiene and are potentially at a higher risk of future oral disease, particularly when their metabolic disorder is uncontrolled. However, factors outside the oral cavity may also have a considerable impact on the initiation and progression of oral diseases.

Key words: *Children, oral health status, Candida albicans*, *Streptococcus mutans*, type 1 diabetes mellitus

INTRODUCTION

Diabetes mellitus (DM), a condition whose primary characteristic is hyperglycaemia, results from defects in insulin secretion, insulin action or both. According to the classification of the American Diabetes Association, among the four types of diabetes, two are prevalent: type 1 diabetes mellitus (T1DM), which is the most common form of diabetes in children (also known as juvenile-onset diabetes); and type 2 diabetes, which is mainly observed in adults[1]. T1DM is characterised by autoimmune β-cell destruction, usually leading to absolute insulin deficiency.

Several studies have looked into the connection between T1DM and the oral health status[2–5]. There is evidence that T1DM has a significant role in the onset and evolution of oral diseases, such as periodontitis[6] and possibly dental caries, as well as in changes of the

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oral microflora which encourage and facilitate the overgrowth of *Candida* spp.[7,8].

Previous studies have demonstrated that adolescents with T1DM have higher prevalence and more severe inflammation of the gingiva than similarly aged healthy individuals, although their plaque levels are comparable. Metabolic imbalances in the tissues may diminish the resistance of persons with diabetes to infection, and thus modify the initiation, development and progression of periodontal disease[7,9–12]. Moreover, it has been proposed that there is a bidirectional relationship between diabetes and periodontal disease, with the presence of periodontitis leading to difficulties in glycaemic control for patients with T1DM[13,14].

On the other hand, evidence on the association between caries and diabetes in children/adolescents is ambiguous. Researchers have described a decreased salivary flow rate in individuals with poorly controlled T1DM[8,15,16] and the subsequent development of dental caries. Children with diabetes are perhaps at a higher risk for caries development than healthy children, mainly because of a lapse in metabolic control, hypofunction of the salivary gland and high concentrations[7] of glucose in their saliva.

Furthermore, studies have also focussed on the oral microbiota of patients with T1DM and the possible changes mediated by the metabolic control status of the disease. Despite the controversy concerning the distribution of periodontopathic bacteria in patients with T1DM[17–20], it is now widely accepted that local conditions in the periodontal pockets of such patients predispose to the growth of Gram-negative bacteria (e.g. *Aggregatibacter actinomycetemcomitans*, *Bacteroides* spp., *Capnocytophaga* spp., *Campylobacter* spp., etc.)[21]. Regarding cariogenic species, some investigators have reported similar levels of salivary mutans streptococci and lactobacilli in subjects with well-controlled diabetes and in healthy controls[22–24], whereas others have suggested a decreased number of these bacteria in patients with diabetes during a period of improved metabolic control[15,25].

Finally, oral fungal infections are more common in adult patients with diabetes than in healthy individuals as a result of the associated immunodeficiency[26,27]. Evidence suggests an increase in the carriage frequency and the amount of *Candida albicans* in subjects with diabetes compared with non-diabetic subjects[26–28]; however, this is not widely confirmed[29]. Some studies have shown that reduced metabolic control, increased concentration of glucose in blood and saliva, long duration of the disease and the presence of complications resulting from diabetes are correlated to increased *Candida* carriage and clinical signs of candidiasis[27,30,31], while other researchers have failed to find such a relationship[32]. For children, a recent study found that T1DM did not confer a higher predisposition for yeast colonisation[33]. The contradictory results and the fact that most of these studies refer to adults justify the investigation of growth patterns of oral *Candida* spp. in children and adolescents with T1DM.

The purpose of this cross-sectional study was to study the oral health status of youngsters with T1DM and different levels of glycaemic control and determine the oral microbial load of oral *C. albicans* and *Streptococcus mutans*, the primary cariogenic species. These diabetic individuals were subsequently compared with an age-matched group of healthy children and adolescents.

METHODS

Study population

The study population comprised a convenience sample of 144 young individuals 6–15 years of age (*Table 1*). The diabetic group included 74 children (31 boys, 43 girls) with T1DM, with a mean diabetes duration of 4.21 ± 2.93 years, all diagnosed and followed at the Diabetes Center of the First Department of Pediatrics of the Medical School of the National and Kapodistrian University of Athens (NKUA) in the 'Aghia Sophia' Children's Hospital, which is the largest public hospital for children in Greece. Their metabolic control classification, while following the prescribed insulin regimen, was based on the glycosylated haemoglobin (HbA1c) test, which reflects the average blood glucose level over the past 2–3 months. The diabetic population was divided into two groups (*Table 1*): T1DM patients with poor glycaemic control (PGC: $n = 35$: 13 boys and 22 girls; HbA1c ≥7.5%) and T1DM patients with good glycaemic control (GGC: $n = 39$: 18 boys and 21 girls; HbA1c <7.5%). The inclusion criteria for the patient groups were as follows:

- Diabetes should have been diagnosed at least 1 year prior to the examination.

Table 1 Characteristics of the three groups of individuals

Study group	Gender	Mean age (SD)	Diabetes duration (years)
C ($n = 70$)	41 girls, 29 boys	11.07 (2.86)	–
GGC ($n = 39$)	21 girls, 18 boys	11.84 (2.66)	4.05 (2.68)
PGC ($n = 35$)	22 girls, 13 boys	12.35 (2.35)	6.15 (3.40)

C, non-diabetic control; GGC, good glycaemic control; PGC, poor glycaemic control.

- The patients should be free of other systemic diseases apart from Hashimoto's thyroiditis (autoimmune disease).
- The patients should not have taken any other medication (including antibiotics over the previous 3 months), apart from that necessary for diabetes mellitus.
- The patients should not be receiving orthodontic treatment.

The control group (C) consisted of 70 healthy non-diabetic youngsters (*Table 1*) without any reported systemic disease and medication use, including the use of antibiotics in the last 3 months. These children were followed or requested dental care in the Dental School of the NKUA. They were from the greater Athens metropolitan region and matched for age, sex and socio-economic status with the T1DM group.

The study was performed in full accordance with the World Medical Association Declaration of Helsinki. Ethical approval was obtained from the 'Research Ethics Committee' of the NKUA School of Dentistry on 31 July 2014 (protocol approval number 247). All patients were included in the study after the written informed consent had been signed by their parents. The recruitment of the patients was carried out between June 2014 and October 2015.

Questionnaire

All individuals completed a questionnaire at baseline which provided information concerning their socio-economic status, oral hygiene and dietary habits as well as frequency of dental care. The socio-economic status was determined based on mother's and father's education (i.e., primary school, secondary school, comprehensive education, high school or university degree). The questions concerning oral hygiene habits provided insight into the frequency of toothbrushing, use of fluoride toothpaste and dental floss. Questions on dietary habits examined the frequency of main meals and the consumption of sweet snacks and drinks.

Clinical intra-oral examination

Clinical intra-oral examination of the diabetic youngsters was performed under standardised dental conditions in the dental clinic at the Children Hospital 'Aghia Sofia', by two calibrated dentists. The inter-examiner agreement was high ($\kappa > 0.90$).

Dental plaque

The presence of dental plaque was scored using the plaque index (PlI) of Silness and Löe[34]. Plaque was registered at the Ramfjord teeth without using a disclosing agent. The mean PlI index for each patient was calculated by adding together the scores for the six different tooth surfaces (mesial labial/buccal, labial/buccal, distal labial/buccal, mesial lingual, lingual, distal lingual) and dividing the summed score by the total number of surfaces examined.

Gingival condition

The gingival condition was assessed at six sites (mesial labial/buccal, labial/buccal, distal labial/buccal, mesial lingual, lingual, distal lingual) of each of the Ramfjord teeth. Each site was given a score from 0 to 3, according to the Löe and Silness gingival index (GI)[35]. The mean score for each patient was calculated by adding together the scores for all tooth surfaces and dividing the summed score by the total number of surfaces examined.

Calculus

The calculus index (CI) scores for the quantity of calculus at the cervix of every tooth were assessed and recorded for all four tooth surfaces (mesial, distal, buccal and lingual)[36,37]. The mean score for each patient was calculated by adding together the scores for all tooth surfaces and dividing the summed score by the total number of surfaces examined.

Dental caries

All teeth, after professional tooth cleaning, were examined using a mouth mirror and a blunt dental explorer. Caries was recorded using the International Caries Diagnosis and Assessment System (ICDAS II) criteria[38], assessing the activity of lesions at both the cavitated and non-cavitated stage. Results are presented using the decayed, missing and filled tooth surface (DMFS) index.

Salivary sampling and microbiological analysis

Paraffin-stimulated whole saliva was collected in the morning, for 5 minutes, at least 1 hour after food intake and immediately before the clinical examination. Flow rate was determined using the CRT test (Ivoclar-Vivadent, Schaan, Liechtenstein) and calculated as ml/minute. Rates of stimulated salivary flow under 0.7 ml/minute suggest xerostomia and flow rates of $\geq$0.7 ml/minute are considered as normal[39]. In addition, 1 ml of saliva was used to calculate the buffer capacity using the CRT buffer capacity test.

For detection and quantification of salivary *Candida* and mutans streptococci, 300 µl of stimulated saliva was transferred to sterile Eppendorf plastic vials (Eppendorf AG, Hamburg, Germany), to which 300 µl of Tris–EDTA (TE) buffer (10 mM Tris–HCl, 1 mM EDTA, pH 7.6) and 300 µl of 1 M sodium hydroxide (NaOH) was added. The samples were

transported to the Laboratory of Microbiology, School of Medicine, NKUA, where they were stored frozen at −80 °C. These samples were used subsequently for the detection and enumeration of oral *C. albicans* and *S. mutans* using real-time polymerase chain reaction (PCR).

Microbial DNA extraction

The saliva samples were processed using the NucleoSpin Tissue kit (Macheray-Nagel, Düren, Germany) following the hard-to-lyse bacteria protocol provided by the manufacturer. Briefly, the samples were centrifuged at 8,000 ***g*** for 5 minutes, the supernatant was removed and the pellet was resuspended in 180 μl of T1 buffer (20 mM Tris–HCl, pH 8.0, 2 mM EDTA, 1% Triton X-100, pH 8) supplemented with 20 mg/ml of lysozyme. The suspension was incubated at 37°C for 1 hour, after which 25 μl of proteinase K was added to every sample which were then incubated overnight at 56°C. After this period of incubation, the protocol followed was as described in the manufacturer's manual. The final elution volume was 60 μl.

Real-time PCR

Real-time PCR was carried out using a Stratagene mx 3005 system (Agilent Technologies, Santa Clara, CA, USA). The PCR protocol was as follows for both pathogens: enzyme activation, 2 minutes at 95°C; denaturation, 10 seconds at 95 °C; and data collection with FAM, 60 seconds at 60 °C. The number of PCR cycles was 40.

In order to construct a standard curve for quantification of the samples, 10-fold serial dilutions of the positive controls provided with the kit were used. The primary positive control for both pathogens was 2×10^5 copies per μl and the last dilution was two copies per μl. The samples were measured in copies per μl by comparison with the Standard Curve and then transformed to copies per ml using the concentration factor, which was 5.

Commercially available probes, provided by Primer Design Ltd. (Southampton, UK), were used for detection of *C. albicans* and *S. mutans*. The *C. albicans* primers and probe have been designed specifically and exclusively for the *in vitro* quantification of all *C. albicans* strains and do not detect other *Candida* species. The target sequence [ribonuclease P RNA (*RPR1*) gene] used in this kit has previously been shown to be a good genetic marker for *C. albicans* in other PCR-based studies. Also, the target sequence for *S. mutans*, the glucosyltransferase-I (*gtfb*) gene, has previously been identified as a highly specific target for the detection of *S. mutans*. The primers and probes of both *S. mutans* and *C. albicans* kits have 100% homology with all reference sequences for *S. mutans* in the NCBI database.

Statistical methods

Descriptive statistics were calculated for study group characteristics. As the data were not normally distributed, median regression was implemented to assess potential associations between the clinical characteristics (PlI, GI, CI, DMFS) of the two diabetes groups and the control group. Microbial counts were log-transformed. All analyses were conducted using Stata 14.1 (StataCorp, College Station, TX, USA). The level of significance was set at 0.05.

RESULTS

Questionnaire

Social status

More than one-quarter (28.6%) of fathers of the children in the PGC group had a low level of education, whereas this was the case only for 7.7% and 4.3% of the fathers of children in the GGC and C groups, respectively (results not shown). A high level of father's education was reported by 25.7% of children in the PGC group, 35.9% in the GGC group and 58.6% in the C group.

Oral hygiene and dietary habits

Analysis of the questionnaire results for oral hygiene habits showed that essentially all (99.8%) participating children were using fluoridated toothpastes. More than half (51.3%) of all children in the GCC group and almost half (45.7%) of all children in the C group brushed their teeth twice a day, compared with only 37.1% of those in the PGC group. Moreover, almost one-third (31.4%) of children in the PGC group did not brush their teeth every day, in contrast to approximately one in 10 children of the other two groups (10.3% in the GGC group and 10% in the C group). Almost 28% of all children brushed their teeth without parental help.

More than two-thirds of children in PGC and GGC groups reported that they had never used dental floss, compared with 41% of children in the C group. Both PGC and GGC groups were similar with respect to frequency of dental visits (almost 34% had dental attendance twice per year), while the respective percentage for the C group was 56%. However, the C group also had an important percentage of subjects (14.2%) who visited the dental office infrequently (less than once every 2 years). There were no significant differences in frequency of the mean sugary

meals and snacks per day among children in the PGC and GGC groups ($P > 0.05$).

Periodontal status

Dental plaque

The dental plaque level (PlI) was found to be significantly different among the three groups. Children in the PGC group had significantly more plaque than those in GGC and C groups ($P = 0.024$ and 0.017, respectively; *Table 2*).

Gingival inflammation

The GI values were not significantly different between the groups ($P > 0.05$). The analysis of this factor as a dichotomous score (i.e., presence or absence of inflammation) also failed to show a significant difference between the groups and was not affected by the duration of diabetes. However, children in the PGC group had more inflammation than those in the other two groups (*Table 2*).

Calculus index

No statistically significant differences in mean CI were found between the groups ($P > 0.05$; *Table 2*).

Dental caries

Although children in the PGC group had a higher DMFS score than children in the other two groups [mean (SD): 3.09 (5.76) *vs*. 1.18 (2.22) and 1.48 (3.37)], no statistical significance was found for these findings among groups. Decayed surfaces comprised the biggest component of the DMFS score in all groups [1.91 (PGC group) *vs*. 0.42 (GGC group) and 0.6 (C group)] ($P > 0.05$; *Table 2*).

Table 2 Caries experience and levels of plaque, gingivitis and calculus in permanent teeth of individuals in the poor glycaemic control (PGC), good glycaemic control (GGC) and non-diabetic control (C) study groups

Variable	PGC	GGC	C	*P*-value
Caries				
DS	1.91 (4.15)	0.42 (1.06)	0.6 (1.98)	>0.05
MS	0.29 (1.69)	0.39 (1.37)	0.15 (0.86)	>0.05
FS	0.89 (2.34)	0.39 (0.97)	0.73 (2.09)	>0.05
DMFS	3.09 (5.76)	1.18 (2.22)	1.48 (3.37)	>0.05
PlI	$0.85^{a,b}$ (0.61)	0.55^{a} (0.57)	0.39^{b} (0.49)	^{a}0.024 ^{b}0.017
GI	0.34 (0.56)	0.23 (0.42)	0.15 (0.41)	>0.05
CI	0.60 (0.79)	0.34 (0.45)	0.29 (0.45)	>0.05

Values are given as mean (SD). Values with the same superscript letters were significantly different when compared (*P*-value shown in last column).
CI, calculus index; DMFS, decayed, missing, filled surface (index); DS, decayed surface; MS, missing surface; FS, filled surface; GI, gingival index; PlI, plaque index.

Salivary parameters

In all children, a normal flow rate [mean (SD): 1.55 (0.6) ml/minute for the PGC group; 1.49 (0.62) ml/minute for the GGC group; and 1.29 (0.46) ml/minute for the C group] and a high buffering capacity were recorded.

Microbiological results

Candida albicans was found in 13 of the 35 children in the PGC group, in nine of the 39 children in the GGC group and in 13 of the 70 children in the C group. The mean values of *C. albicans* were approximately 3 log colony-forming units (CFUs)/ml among the three groups (*zTable 3*). *Streptococcus mutans* was isolated from the oral cavity of 96 (67%) of the 144 individuals. A statistically significant difference in the levels of *S. mutans* was found between the PGC group and the C group ($P = 0.032$). *Streptococcus mutans* was detected in 77% of children in the PCG group, 74% of children in the GGC group and 57% of children in the C group. The mean values of *S. mutans* are presented in *Table 3*.

DISCUSSION

Children with T1DM face numerous health challenges in their life, including dental and oral diseases. In the present research, the impact of diabetes on overall oral health was evaluated using both clinical and microbiological findings.

The results presented here indicate that the prevalence and severity of dental caries, a true oral-health

Table 3 Prevalence of *Candida albicans* and *Streptococcus mutans* in individuals of the poor glycaemic control (PGC), good glycaemic control (GGC) and non-diabetic control (C) study groups

Microbial species	PGC	GGC	C
C. albicans			
Detection frequency	13/35	9/39	13/70
Mean (SD) log number	3.01 (0.94)	2.87 (0.92)	3.13 (1.14)
95% confidence interval	2.44–3.58	2.16–3.57	2.44–3.82
S. mutans			
Detection frequency	27/35	29/39	40/70
Mean (SD) log number	5.47 (1.05)*	4.70 (1.82)	4.29 (1.55)*
95% confidence interval	5.05–5.89	4.02–5.38	3.82–4.86

C, control, individuals; GGC, good glycaemic control; PGC, poor glycaemic control.
*Statistical significance ($P = 0.032$) for *S. mutans*: PGC *vs*. C.

end point[40], are similar in both children with T1DM and age-matched healthy individuals. This finding confirms previous studies in diabetic children[3,5]. Similar dietary and toothbrushing habits among diabetic and healthy study participants contributed to this finding. However, a study by Svensson *et al.*[4] found a difference in caries risk between diabetic and healthy individuals. They attributed their findings to the related xerostomia or the decreased salivary flow rate associated with hyperglycaemia and the lack of preventive and regular dental care. The multifactorial aetiology of caries, the levels of glucose in saliva and gingival crevicular fluid, as well as the onset of the disease influence the caries risk in diabetic children and consequently may explain the contradictions found in the literature[7,15,41].

In the present study, participants with poorly controlled T1DM showed significantly more dental plaque, which, along with calculus may increase the risk for gingival inflammation (the second true end point of the present study). A similar finding was supported by Dakovic and Pavlovic[2] who studied periodontal disease in Serbian children and adolescents with T1DM. They showed a higher prevalence of periodontal disease in youngsters with T1DM compared with their healthy counterparts and that there was an association between the overall duration of the condition and the level of metabolic control with the severity of gingival inflammation. By contrast, in the diabetic children in the present study, no association was found between duration of diabetes, which was statistically different for the two diabetes groups, and gingival inflammation levels (as measured by the GI). This was true even if inflammation was evaluated on a simplified dichotomous scale (i.e., presence or absence of inflammation).

Although, in the present study, more plaque was found in children from the PGC group, no differences were found for GI (representing the second true end point of inflammation) between the groups of participants. Thus, from the findings in the present group of individuals there was no clear effect of glycaemic control on the level of gingival inflammation. This is in contrast to other studies, which found that diabetic children have significantly higher gingival bleeding index scores than the healthy population[7,42–47]. Nevertheless, one should bear in mind that in the current analysis, most of the individuals with TIDM had HbA1c values between 5.9% and 9% (only 7 of 74 patients had an HbA1c value above 9%), indicating relatively good metabolic control for the patients in the pediatric diabetes clinic. This finding together with the young age of the patients, may be responsible for the failure to find differences in gingival inflammation. Although no significant differences were found between groups, diabetic children with poor glycaemic control showed a clear tendency to have higher GI scores than diabetic children with good glycaemic control and healthy individuals[7,48–50]. Therefore, the significantly higher amount of plaque and the higher GI in children with poorly controlled diabetes indicates that there may be an increased risk for periodontal disease in this group. The presence of these classic risk factors (increased PlI and GI) in adolescence may indeed lead to significant disease in future adult life, but this cannot be readily proven. Long-term studies are essential if factors which are present during childhood are to be investigated in relation to development, or progression, of a disease (such as periodontitis) during adulthood.

Differences in the oral microbiota between diabetic and healthy individuals may significantly influence the incidence of oral diseases caused by bacteria (e.g., periodontal disease and dental caries). Observations confirm that in individuals with diabetes, including adolescents, there are differences in the levels of periodontal pathogens (both in the type and their overall number), their habitat and the age that disease manifests when compared with the healthy population[51,52]. Others attribute increased disease severity to a hyperinflammatory response in diabetic individuals, without there necessarily being a significant difference in the microbial composition when compared with non-diabetic individuals[53]. Concerning the levels of cariogenic species, which are considered as surrogate measures in caries research[54], the present findings are inconclusive. Reports mention both insignificant differences in the levels of *S. mutans* and *Lactobacilli* in healthy and diabetic individuals[3,5,52] but also a higher proportion of diabetic individuals with elevated levels of *S. mutans* in their saliva compared with controls. In the present study, a statistically significant difference in the levels of *S. mutans* was found between the PGC group and the control group ($P = 0.032$). *Streptococcus mutans* was detected in 75% of children with T1DM. More specifically, 77% of PGC diabetic children, 74% of GGC diabetic children and 57% of healthy controls were positive for *S. mutans*.

Regarding the levels of *Candida* spp. in the oral cavity, there are reports of not only no differences between diabetic and healthy children[3,5,33], but also of higher levels in diabetic individuals *versus* the healthy population[16]. Although diabetes was found to be a predisposing factor for candidiasis[55,56], the findings in the literature are inconsistent. While an increase in *Candida* density was accompanied with increased concentration of salivary glucose[57–59], no statistically significant differences in frequency and the number of yeasts (CFUs/ml) were found between diabetic and healthy patients by Darwazeh *et al.*[60] In that study, the young age of the patients and the limited number of participants with very poor glucose

levels in blood samples may be connected to the similar amounts of *C. albicans* carried in all the study participants. In our study, approximately one-third of all diabetic patients were positive for *C. albicans*. However, individuals in the PGC group were more frequently positive (37%) for *C. albicans* than individuals in the GGC (23%) and C (19%) groups. The mean (SD) values of *C. albicans* were comparable between groups: 3.01 (0.94) log CFUs/ml in the PGC group; 2.87 (0.92) in the GGC group; and 3.13 (1.19) in the C group. The somewhat lower level of *C. albicans* carriage in the present study in comparison with that reported in other studies, may be due to differences in methodology. The present study utilised real-time PCR to detect and enumerate *C. albicans*, while the majority of previous studies in general used classical microbiology to detect yeasts.

With respect to the inconclusive nature of the findings when comparing the current study with other studies, more research is needed to clarify the potential differences between non-diabetic and diabetic individuals (children, young adults and adults) in terms of their oral health in the long-term and other predisposing genetic factors not strictly located within the oral cavity. A recommendation for future studies would be also to measure the salivary glucose levels and correlate those with the levels of bacteria, something not performed here. Moreover, whether the increase in plaque levels and *S. mutans* levels are a result of the glycaemic control or indicative of general shortcomings in health behaviours of the individuals remains unanswered.

An important factor that may impact the oral health findings is the frequency of dental attendance of the individuals. A larger proportion of individuals in the C group reported a higher frequency (twice per year) of visits to the dental office (55.7%), compared with individuals in PGC (34.3%) and GGC (33.3%) groups. This could possibly be considered to impact on the oral health status and the ensuing comparisons, and thus be a source of bias. However, this difference is more than offset by the proportion of individuals in the C group (14.2%) who rarely went to the dentist (less than one visit per 2 years). Additionally, it must be noted that this concerns the reported behaviour of the individuals and as such may describe a desired outcome but not what occurs in reality.

In conclusion, although there seems to be a tendency for greater plaque accumulation (quantitative changes) and *S. mutans* colonisation (qualitative changes), no clear differences in colonisation with specific pathogens, such as *C. albicans* (qualitative changes), were detected from the saliva samples of diabetic individuals when compared with matched controls, in the age category examined. An increased risk for oral diseases could be presumed based on the cumulative effect of a compromised oral environment and on other factors, such as age of onset and metabolic control of diabetes, rather than purely the increased presence of specific pathogens. Thus, the children with poor glycaemic control may be regarded as having a higher risk for future dental disease in comparison with children with well-controlled T1DM. In addition, as the levels of HbA1c can change and the genetic factors impacting susceptibility have yet to be fully elucidated, all diabetic children should have regular dental visits. Future research should focus on more in depth investigation on the association of the various susceptibility factors (e.g., inflammatory response) linked to diabetes as well as the role of glycaemic control over the years with the oral health status and microbiome composition.

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Conflict of interest

All authors declare that they have no conflict of interest to disclose.

REFERENCES

1. American Diabetes Association. 2. Classification and diagnosis of diabetes: standards of medical care in diabetes-2018. *Diabetes Care* 2018 41 (Suppl 1): S13–S27.
2. Dakovic D, Pavlovic MD. Periodontal disease in children and adolescents with type 1 diabetes in Serbia. *J Periodontol* 2008 79: 987–992.
3. Siudikiene J, Machiulskiene V, Nyvad B *et al.* Dental caries increments and related factors in children with type 1 diabetes mellitus. *Caries Res* 2008 42: 354–362.
4. Svensson K, Perez CM, Suarez EL *et al.* Dental caries and diabetes in the elderly population in San Juan, Puerto Rico. Presented at: 137th Annual Meeting and Exhibition, American Public Health Association; November 11, 2009; Philadelphia, Pennsylvania.
5. El-Tekeya M, El Tantawi M, Fetouh H *et al.* Caries risk indicators in children with type 1 diabetes mellitus in relation to metabolic control. *Pediatric Dent* 2012 34: 510–516.
6. EFP Manifesto. Perio and general health. Available from: https://www.efp.org/efp-manifesto/EFP_manifesto_full_version_2016.pdf. Accessed 22 November 2017.
7. Karjalainen J, Knuttila MLE. The onset of diabetes and poor metabolic control increases gingival bleeding in children and adolescents with insulin-dependent diabetes mellitus. *J Clin Periodontol* 1996 23: 1060–1066.
8. Arheiam A, Omar S. Dental caries experience and periodontal treatment needs of 10- to 15-year old children with type 1 diabetes mellitus. *Int Dent J* 2014 64: 150–154.
9. Löe H. Periodontal disease: the sixth complication of diabetes mellitus. *Diabetes Care* 1993 16: 329–334.
10. Papapanou PN. Periodontal diseases: epidemiology. *Ann Periodontol* 1996 1: 1–36.

11. Mealey B. Diabetes and periodontal diseases. *J Periodontol* 1999 70: 935–949.
12. Taylor GW. Bidirectional interrelationships between diabetes and periodontal diseases: an epidemiologic perspective. *Ann Periodontol* 2001 6: 99–112.
13. Nagata T. Relationship between diabetes and periodontal disease. *Clin Calcium* 2009 19: 1291–1298.
14. Mirza BA, Syed A, Izhar F *et al*. Bidirectional relationship between diabetes and periodontal disease: review of evidence. *J Pak Med Assoc* 2010 60: 766–768.
15. Twetman S, Nederfors T, Stahl B *et al*. Two-year longitudinal observation of salivary status and dental caries in children with insulin-dependent diabetes mellitus. *Pediatric Dent* 1992 14: 184–188.
16. Siudikiene J, Machiulskiene V, Nyvad B *et al*. Dental caries and salivary status in children with type 1 diabetes mellitus, related to the metabolic control of the disease. *Eur J Oral Sci* 2006 114: 8–14.
17. Sastrowijoto SH, Hillemans P, van Steenbergen TJ *et al*. Periodontal condition and microbiology of healthy and diseased periodontal pockets in type 1 diabetes mellitus patients. *J Clinl Periodontol* 1989 16: 316–322.
18. Pinducciu G, Micheletti L, Piras V *et al*. Periodontal disease, oral microbial flora and salivary antibacterial factors in diabetes mellitus type 1 patients. *Eur J Epidemiol* 1996 12: 631–636.
19. Grossi SG, Skrepcinski FB, DeCaro T *et al*. Treatment of periodontal disease in diabetics reduces glycated hemoglobin. *J Periodontol* 1997 68: 713–719.
20. Salvi GE, Kandylaki M, Troendle A *et al*. Experimental gingivitis in type 1 diabetics: a controlled clinical and microbiological study. *J Clin Periodontol* 2005 32: 310–316.
21. Sharma M, Tiwari SC, Singh K *et al*. Occurrence of bacterial flora in oral infections of diabetic and non-diabetic patients. *Life Sci Med Res* 2011 1–6.
22. Swanljung O, Meurman JH, Torkko H *et al*. Caries and saliva in 12-18-year-old diabetics and controls. *Scand J Dent Res* 1992 100: 310–313.
23. Canepari P, Zerman N, Cavalleri G. Lack of correlation between salivary *Streptococcus mutans* and lactobacilli counts and caries in IDDM children. *Minerva Stomatol* 1994 43: 501–505.
24. Karjalainen KM, Knuuttila ML, Käär ML. Relationship between caries and level of metabolic balance in children and adolescents with insulin-dependent diabetes mellitus. *Caries Res* 1997 31: 13–18.
25. Tenovuo J, Lehtonen OP, Viikari J *et al*. Immunoglobulins and innate antimicrobial factors in whole saliva of patients with insulin-dependent diabetes mellitus. *J Dent Res* 1986 65: 62–66.
26. Vazquez JA, Sobel JD. Fungal infections in diabetes. *Infect Dis Clin North Am* 1995 9: 97–116.
27. Guggenheimer J, Moore PA, Rossie K *et al*. Insulin-dependent diabetes mellitus and oral soft tissue pathologies: II. Prevalence and characteristics of Candida and Candidal lesions. *Oral Sur Oral Med Oral Path Oral Radio Endodont* 2000 89: 570–576.
28. Aly FZ, Blackwell CC, Mackenzie DA *et al*. Factors influencing oral carriage of yeasts among individuals with diabetes mellitus. *Epidemiol Infect* 1992 109: 507–518.
29. Kadir T, Pisiriciler R, Akyüz S *et al*. Mycological and cytological examination of oral candidal carriage in diabetic patients and non-diabetic control subjects: thorough analysis of local aetiologic and systemic factors. *J Oral Rehabil* 2002 29: 452–457.
30. Al-Maskari AY, Al-Maskari MY, Al-Sudairy S. Oral manifestations and complications of diabetes mellitus: a review. *Sultan Qaboos Uni Med J* 2011 11: 179–186.
31. Shenoy MP, Puranik RS, Vanaki SS *et al*. A comparative study of oral candidal species carriage in patients with type1 and type2 diabetes mellitus. *J Oral Maxillofac Path* 2014 18(Suppl 1): S60–S65.
32. Fisher BM, Lamey PJ, Samaranayake LP *et al*. Carriage of Candida species in the oral cavity in diabetic patients: relationship to glycaemic control. *J Oral Path* 1987 16: 282–284.
33. Costa AL, Silva BMA, Soares R *et al*. Type 1 diabetes in children is not a predisposing factor for oral yeast colonization. *Med Mycol* 2017 55: 358–367.
34. Silness J, Löe H. Periodontal disease in pregnancy. II. Correlation between oral hygiene and periodontal condition. *Acta Odontol Scand* 1964 22: 121–135.
35. Löe H, Silness J. Periodontal disease in pregnancy. I. Prevalence and severity. *Acta Odontol Scand* 1963 21: 533–551.
36. Greene JC, Vermillion JR. The oral hygiene index: a method for classifying oral hygiene status. *J Am Dent Assoc* 1960 61: 172–179.
37. Greene JC, Vermillion JR. The Simplified oral hygiene index. *J Am Dent Assoc* 1964 68: 7–13.
38. ICCMS™ Guide for Practitioners and Educators. Available from: https://www.iccms-web.com/uploads/asset/592845add7ac8756944059.pdf. Accessed 8 June 2018.
39. Navazesh M, Kumar SK. Measuring salivary flow: challenges and opportunities. *J Am Dent Assoc* 2008 139(Suppl): 35S–40S.
40. Hujoel PP. Endpoints in periodontal trials: the need for an evidence-based research approach. *Periodontol 2000* 2004 36: 196–204.
41. Taylor GW, Manz MC, Borgnakke WS. Diabetes, periodontal diseases, dental caries, and tooth loss: a review of the literature. *Compend Cont Edu Dent* 2004 25: 179–184, 186–188, 190.
42. Siudikiene J, Maciulskiene V, Dobrovolskiene R *et al*. Oral hygiene in children with type I diabetes mellitus. *Stomatologija* 2005 7: 24–27.
43. Lalla E, Cheng B, Lal S *et al*. Periodontal changes in children and adolescents with diabetes: a case-control study. *Diabetes Care* 2006 29: 295–299.
44. Lalla E, Cheng B, Lal S *et al*. Diabetes mellitus promotes periodontal destruction in children. *J Clin Periodontol* 2007 34: 294–298.
45. Orbak R, Simsek S, Orbak Z *et al*. The influence of type-1 diabetes mellitus on dentition and oral health in children and adolescents. *Yonsei Medl J* 2008 49: 357–365.
46. López del Valle LM, Ocasio-López C. Comparing the oral health status of diabetic and non-diabetic children from Puerto Rico: a case-control pilot study. *P R Health Sci J* 2011 30: 123–127.
47. Al-Khabbaz AK, Al-Shammari KF, Hasan A *et al*. Periodontal health of children with type 1 diabetes mellitus in Kuwait: a case-control study. *Med Princ Pract* 2013 22: 144–149.
48. Hartsfield JK Jr. Premature exfoliation of teeth in childhood and adolescence. *Adv Pediatr* 1994 41: 453–470.
49. Pinson M, Hoffman WH, Garnick JJ *et al*. Periodontal disease and type I diabetes mellitus in children and adolescents. *J Clin Periodontol* 1995 22: 118–123.
50. Moore PA, Zgibor JC, Dasanayake AP. Diabetes. *J Am Dent Assoc* 2003 134(Suppl): 11S–15S.
51. Dusková J, Broukal Z. [Compensation criteria of basal disease in the prevention and treatment of periodontal disease in patients with diabetes mellitus]. *Praktické zubní lékařství* 1991 39: 51–54.
52. Thorstensson H. Periodontal disease in adult insulin-dependent diabetics. *Swed Dent J Suppl* 1995 107: 1–68.
53. Lalla E, Papapanou PN. Diabetes mellitus and periodontitis: a tale of two common interrelated diseases. *Nat Rev Endocrinol* 2011 7: 738–748.

54. Lo EC, Chu CH, Lin HC. A community-based caries control program for pre-school children using topical fluorides: 18-month results. *J Dent Res* 2001 80: 2071–2074.

55. Hostetter MK. Handicaps to host defense. Effects of hyperglycemia on C3 and *Candida albicans*. *Diabetes* 1990 39: 271–275.

56. Bader MS, Lai SM, Kumar V *et al*. Candidemia in patients with diabetes mellitus: epidemiology and predictors of mortality. *Scand J Infect Dis* 2004 36: 860–864.

57. Knight L, Fletcher J. Growth of *Candida albicans* in saliva: stimulation by glucose associated with antibiotics, corticosteroids, and diabetes mellitus. *J Infect Dis* 1971 123: 371–377.

58. Odds FC, Evans EG, Taylor MA *et al*. Prevalence of pathogenic yeasts and humoral antibodies to candida in diabetic patients. *J Clin Path* 1978 31: 840–844.

59. Willis AM, Coulter WA, Fulton CR *et al*. Oral candidal carriage and infection in insulin-treated diabetic patients. *Diabet Med* 1999 16: 675–679.

60. Darwazeh AM, MacFarlane TW, McCuish A *et al*. Mixed salivary glucose levels and candidal carriage in patients with diabetes mellitus. *J Oral Path Med* 1991 20: 280–283.

Correspondence to:
Sotiria Gizani,
Department of Paediatric Dentistry
Faculty of Dentistry
National and Kapodistrian University of Athens
2 Thivon Street,
115 27 Athens, Greece.
Email: stgizani@dent.uoa.gr