ORIGINAL RESEARCH

Serum levels of inflammatory markers and HbA1c in patients with type 2 diabetes and apical periodontitis: Preliminary findings

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Keywords
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Abstract

This pilot study assessed the glycaemic control and the serum levels of inflammatory mediators in type 2 diabetes (T2DM) patients with apical periodontitis (AP). Thirty individuals were divided into four groups: Healthy (H); with AP (AP); with T2DM (T2DM); and with T2DM and AP (T2DM-AP). Demographic and pharmacological data were registered. The body mass index (BMI) and the levels of glycated haemoglobin (HbA1c) and IL-1β, IL-6, IL-10, CCL3 and CCL4 were evaluated. AP areas were determined radiographically. Mean age was 64 ± 12 years, with 63% females. Most T2DM patients were under treatment with metformin and antihypertensives. BMI and H1bAc were significantly higher in T2DM patients in relation to H and AP groups. The AP areas were larger in the T2DM-AP group, compared with the AP group. These preliminary findings suggest no influence of AP on glycaemic control or inflammatory levels amongst T2DM patients, although T2DM increased the AP severity.

Introduction

Type 2 diabetes mellitus (T2DM) is a chronic non-communicable disease related to the progressive loss of pancreatic β-cell function, leading to insulin resistance and hyperglycaemia. The latest report of the International Diabetes Federation (IDF) estimated global prevalence of 9.3% for T2DM, which corresponds to a half-billion people. This estimation could rise to 10.9%, reaching 700 million affected individuals until 2045. The constant increase in the incidence of T2DM has been broadly related to lifestyle (1).

The pathophysiological mechanisms of T2DM have been elucidated during the last decades. Metabolic alterations lead to the activation of JNK MAP-kinase and nuclear factor-κB (NF-κB) pathways, with an up-regulation of proinflammatory cytokines (2). Circulating bacterial lipopolysaccharide (LPS) triggers inflammation, insulin resistance, glucose intolerance and obesity (3). Sequentially, obesity alters the intestinal microbiota (4), with an increase in gut permeability, allowing the uptake of macromolecules such as LPS (5). The metabolic endotoxaemia underlies a chronic low-grade inflammatory status and insulin resistance, which are both hallmarks of T2DM (6).

It is well recognised that diabetes greatly affects the oral cavity health, causing delayed healing of the oral mucosa, xerostomia, candidiasis, increased incidence and severity of caries, pulp diseases, root canal infections, apical periodontitis (AP) and periodontitis (7,8). The link between T2DM and periodontitis has been the target of several studies (9). A recent meta-analysis concluded that periodontitis has a significant impact on diabetes control, incidence and its related complications. Accordingly, an

association between periodontitis and impaired glycaemic control in healthy individuals has been suggested. Moreover, periodontitis likely accounts for diabetes-related complications and patients with periodontitis are predisposed to develop T2DM (10).

AP is an inflammatory disorder affecting the periapical tissues surrounding the apex of the tooth. It is usually a consequence of untreated deep caries and pulp tissue necrosis, caused by microorganisms colonising the root canal system. Although pain and re-worsening of symptoms may occur, AP is a chronic infection that frequently remains asymptomatic (11). The global prevalence of AP was reported as 52% and 5%, at the individual and the tooth level respectively (12). Of note, it was demonstrated that AP prevalence was higher in patients with T2DM, especially in those with poor glycaemic control (13).

Both periodontitis and AP are inflammatory conditions triggered by similar microbial pathogens (often Gram-negative anaerobic bacteria), with increased circulating levels of cytokines and LPS (14,15). However, there is scarce clinical evidence assessing the relationship between AP and T2DM. Thus, it is tempting to suggest that AP might aggravate the existing inflammation in T2DM, having negative impacts on glycaemic control. Based on the abovementioned evidence, this clinical study aimed to evaluate the glycaemic control of patients with AP and T2DM, correlating these findings with serum levels of inflammatory markers.

Material and methods

Study design and ethical issues

The research protocol of this case–control observational study was approved by the Institutional Ethics and Research Committee (CAAE#46278114.3.0000.5336). Written informed consent was obtained from all participants. All data were deidentified before the analysis, to protect the anonymity of participants. This study conforms to the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines (16).

Study population and inclusion criteria

Thirty adult individuals were recruited from the dental care units of the School of Health and Life Sciences (PUCRS; Brazil). Consecutive patients from the university dental clinics were evaluated from April to December 2019. The definition criteria of cases comprised individuals presenting with both T2DM and at least one tooth with AP (T2DM-AP group) ($n = 6$). Controls were matched by age, and included patients allocated to the following control groups: healthy individuals (H) ($n = 7$); non-diabetic patients diagnosed with at least one tooth with AP ($n = 7$); patients diagnosed with T2DM and free of AP ($n = 10$).

Definition of the main exposures T2DM and AP

The main exposure variables were the presence of T2DM and AP. For T2DM, patients were screened through self-reported medical history and current use of oral anti-diabetes agents or insulin (17). All selected individuals underwent clinical oral examination and a panoramic radiography. In cases of AP suspicion, a periapical radiograph was performed. Patients with teeth that responded negatively to the cold pulp sensibility test (−50°C), and had periapical radiolucency suggestive of AP, were included in the study. Radiographic diagnosis of AP was further confirmed by two experienced endodontists, considering the periapical indexes from 3 to 5, as described by Ørstavik *et al.* (18): 3, changes in bone structure with some mineral loss; 4, apical periodontitis with well-defined radiolucent area and 5, severe apical periodontitis with exacerbating features. Root canal treatment was performed at no cost to the patient when indicated (for participants in the AP and T2DM-AP groups).

Definition of confounding variables

The following sociodemographic data were collected from self-report: age, -gender, years of formal education, marital status and family income in Brazilian Reais (R$). Medical covariables were based on self-reported data and included T2DM duration (in years), body mass index (BMI) – calculated dividing the weight by the height squared (19) (dichotomised as ≤25 or >25), smoking (never vs. current or former) and alcohol consumption (yes or no). Dental covariables included the number of natural teeth (dichotomised as ≤20 or >20) and the number of teeth with AP (dichotomised as ≤1 or >1). Pharmacological data were collected from self-report and included the use of oral hypoglycaemic drugs (categorised in zero, 1 or 2 drugs), insulin (yes or no), antihypertensive drugs (yes or no), lipid-lowering drugs (yes or no), other drugs (yes or no). Additional comorbidities were registered.

Definition of outcome variables, blood sample collection and analysis

The main outcomes were the serum levels of inflammatory markers (IL-1β, IL-6, IL-10, CCL3 and CCL4) and the HbA1c levels. The total blood and serum were collected for determining HbA1c and inflammatory mediators respectively. The samples were stored at −80°C until use.

HbA1c was evaluated by an immunoturbidimetric assay (reference number 385, LabTest, Lagoa Santa, MG, Brazil). After haemolysis, the samples were submitted to the procedures recommended by the manufacturer and read at 600 and 800 nm. Internal controls (5.4% and 11.6%; reference number 303, LabTest, Lagoa Santa, MG, Brazil) and a 5-point calibration curve (4.3–13.3%; reference number 386, LabTest, Lagoa Santa, MG, Brazil) were used. This method is certified by the National Glycohemoglobin Standardization Program (NGSP), and traceable to the Diabetes Control and Complications Trial (DCCT) (20).

The serum levels of cytokines and chemokines were analysed by sandwich enzyme-linked immunosorbent assay (ELISA) by using DuoSet kits, according to the manufacturer's instructions. The following kits were used: human IL-1β/IL-1F2 (DY201-05; 3.91–250 pg mL^{-1}); human IL-6 (DY206-05; 9.38–600 pg mL^{-1}); human IL-10 (DY217B-05; 31.2–2.000 pg mL^{-1}); human CCL3/MIP-1α (DY270-15; 7.81–500 pg mL^{-1}); human CCL4/MIP-1β (DY270-15; 15.6–1000 pg mL^{-1}), all from R&D Systems (Minneapolis, MN, USA).

A secondary outcome variable was the size (area) of the AP lesions. An experienced and calibrated endodontist (intraclass correlation coefficient = 0.99) evaluated the radiographic images at two distinct time-points with a 3-week interval. The interpretation was carried out in a darkened room. The images were exported to ImageJ 1.52p software (National Institutes of Health, Bethesda, MD, USA), and the AP area was measured (in mm^2) using the freehand selection tool, after calibrating the scale of the software. In participants presenting ≥1 teeth with AP, the sum of the total area of all teeth with AP was considered.

A diagrammatic representation of the full protocols used in the present study is presented in Figure 1.

Statistical analysis

Data normality was checked by Shapiro–Wilk test. Statistical analysis was performed by Kruskal–Wallis (non-parametric data), or one-way ANOVA (parametric data). When the interaction of factors was statistically significant, with P values <0.05, further comparisons were conducted by using Dunn's or Tukey's post hoc tests respectively. Frequency data were analysed by the chi-square test for trends. The tests were performed using GraphPad Software® version 8.0.2 (GraphPad Software Inc., San Diego, CA, USA).

Results

Table 1 shows the sociodemographic, dental and medical data of the participants included in this study. There was a greater proportion of females, except in the AP group. The mean age was 64 ± 11.82 years (mean ± SD), ranging from 39 to 84 years. A statistical comparison of the mean ages of the four groups (one-way ANOVA, followed by Tukey's test) did not reveal any significant difference ($P > 0.05$). The H group presented an upper percentage of married individuals and higher family incomes, although the values were not significantly different, in comparison with the other groups. The duration of T2DM was similar, regardless of AP diagnosis. The percentages of patients with BMI >25 did not significantly differ among groups, according to the analysis by the Chi-square test. Smoking and alcohol habits did not exhibit great differences. In the T2DM group, the number of missing teeth was significantly higher, in relation to the H and AP groups (one-way ANOVA, followed by Tukey's test; $P < 0.01$). The numbers of teeth presenting AP, or the number of patients with more than one AP lesion, were not significantly different, irrespective of the T2DM diagnosis (Chi-square test; $P > 0.05$).

All patients from the T2DM and T2DM-AP groups were under treatment with the anti-diabetic drug metformin, except one that had a later diagnosis of T2DM. In the T2DM group, one patient referred the use of metformin plus glibenclamide. In the T2DM-AP group, two patients used metformin combined with glimepiride or alogliptin. Alternatively, other three patients in the T2DM group used insulin in association with metformin, whereas only one in the T2DM-AP group used this combination. The number of patients under treatment with antihypertensive and/or lipid-lowering drugs were significantly higher in T2DM, independent of AP diagnosis (Chi-square test; $P < 0.01$).

Data depicted in Figure 2a show that BMI was generally higher in T2DM and T2DM-AP groups, although only the T2DM group without AP presented significantly higher BMI values (one-way ANOVA, followed by Tukey's test; $P < 0.05$), in comparison with the H group. The H1bAc was significantly higher in individuals allocated in the T2DM group, when compared with the H and AP groups (Kruskal–Wallis, followed by Dunn's test; $P < 0.05$; $P < 0.01$ respectively). Despite the absence of significant differences, the mean H1bAc was also higher in the T2DM-AP group, in relation to the respective controls (Figure 2b). Half of the patients with type 2 diabetes had H1bAc values greater than 10%, well above the recommended limits for diabetes control.

The evaluation of serum cytokines showed undetectable levels of the pro-inflammatory cytokine IL-6 in either of the groups (Figure 3a). Only one patient in the AP group presented detectable levels of IL-1β (Figure 3b). The anti-inflammatory cytokine IL-10 was identified in the serum of three patients, irrespective of the experimental group (Figure 3c). Two patients in the AP

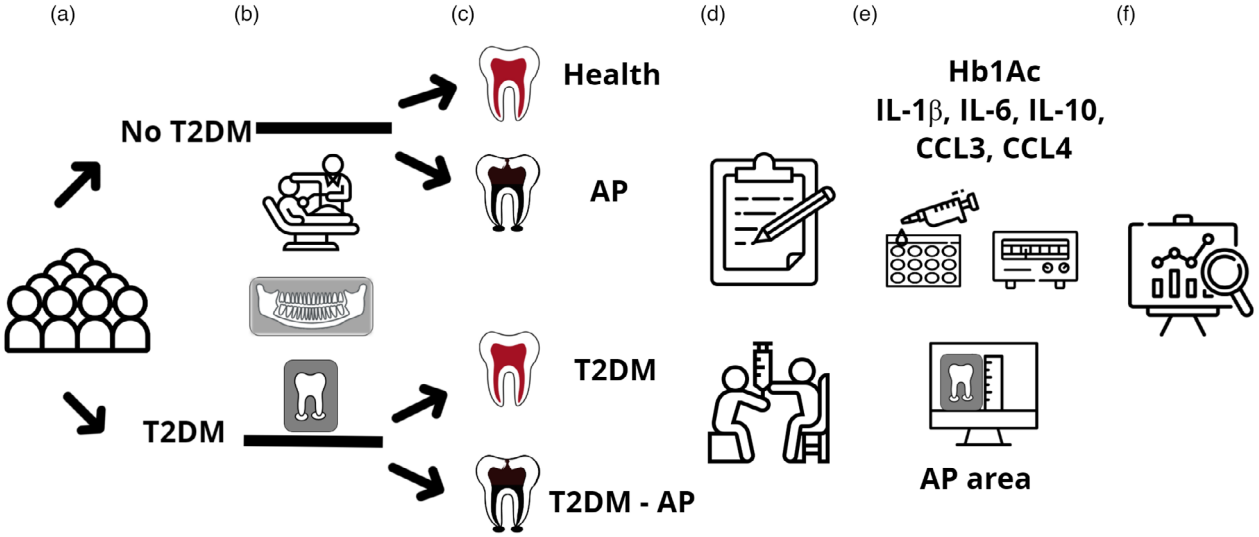

Figure 1 Study Design (a) Patients were classified as healthy (no T2DM) or T2DM, based on self-reported medical history. (b) Screening for AP was performed by clinical and radiographic examinations. (c) One case group (T2DM-AP) and three age-matched control groups were enrolled in the study: Heathy (H), AP and T2DM. (d) Sociodemographic and medical data were registered and blood samples were collected. (e) Hb1AC was measured by immunoturbidimetric assay, and inflammatory mediators by ELISA; AP area was measured by Image J. (f) Statistical data analysis.

Table 1 Sociodemographic, medical, dental and pharmacological characteristics of the sample

	No diabetes		Diabetes	
Variable	H $n = 7$	AP $n = 7$	T2DM $n = 10$	T2DM-AP $n = 6$
Socio-demographic				
Age (year)	59.3 ± 14.4	59.3 ± 9.6	68.8 ± 7.2	58.7 ± 9.0
Gender (Female)	5 (71.4%)	2 (28.6%)	8 (80%)	4 (66.7%)
Education (≥11 years)	6 (85.7%)	6 (85.7%)	6 (60%)	5 (83.3%)
Marital Status (Marriage)	5 (71.4%)	3 (42.8%)	4 (40%)	3 (50%)
Mean Family Income (R$)	8.571,43	3.228,00	4.878,00	2.647.67
Medical				
Diabetes duration (years)	–	–	8.2 ± 9.2	7 ± 2.9
BMI (>25)	3 (42.9%)	5 (71.4%)	9 (90%)	5 (83.3%)
Smoking (current or former)	5 (71.4%)	3 (42.8%)	4 (40%)	4 (66.7%)
Alcohol	0	1 (14.3%)	1 (10%)	1 (16.7%)
Dental				
Number of teeth (median)	27	21	12.5*,**	21
Number of teeth (>20)	5 (71.4%)	4 (40%)	1 (10%)	3 (50%)
Number of teeth with AP (median)	–	1	–	2
AP (>1)	–	3 (42.8%)	–	5 (83.3%)
Drugs				
Oral hypoglycaemic (one drug)	0	0	8 (80%)	5 (83.3%)
Oral hypoglycaemic (two drugs)	0	0	1 (10%)	2 (33.3%)
Insulin	0	0	3 (30%)	1 (16.7%)
Antihypertensive	2 (28.6%)	1 (14.3%)	9 (90%)	6 (100%)***
Lipid-lowering	0	0	7 (70%)	3 (50%)***
Others	3 (42.8%)	2 (28.6%)	5 (50%)	2 (33.3%)

*,**$P < 0.05$, $P < 0.01$; T2DM vs. H and AP respectively (One-way ANOVA followed by Tukey). ***$P < 0.01$ (Chi-square test for trends). Data are presented as median, mean ± SD or *n* (%), AP, apical periodontitis; BMI, body mass index; R$, Brazilian Reais.

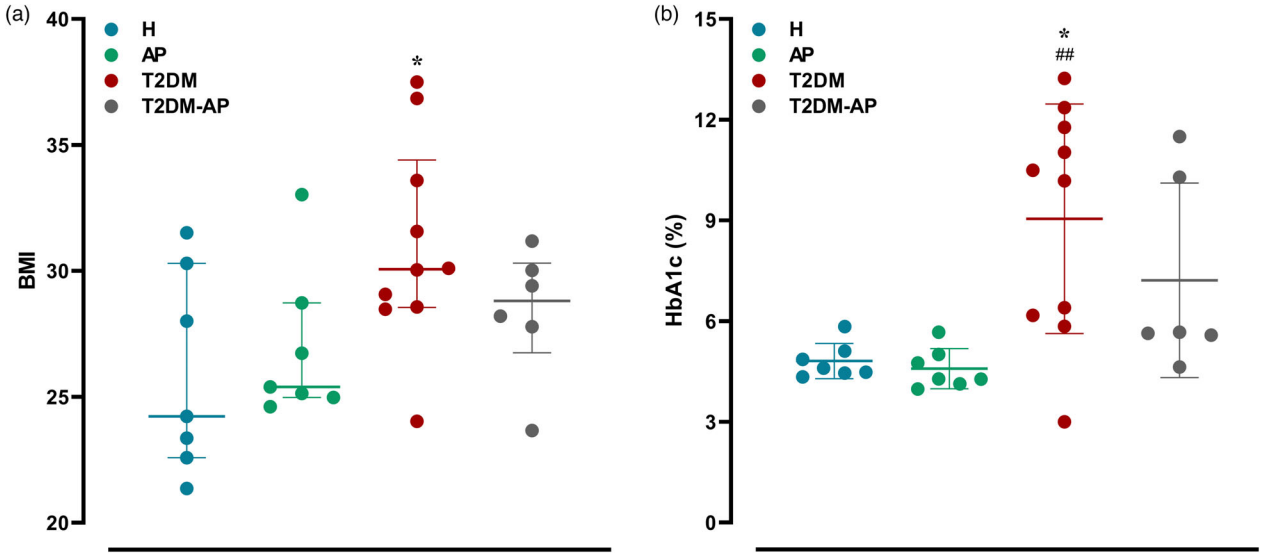

Figure 2 Comparison of (a) body mass index (BMI) and (b) glycated haemoglobin (Hb1Ac) in healthy controls (H), patients with apical periodontitis (AP), individuals with type 2 diabetes diagnosis (T2DM) or patients with both conditions (T2DM-AP). One-way ANOVA revealed a significant difference between H versus T2DM for BMI. Kruskal–Wallis followed by Dunn's test revealed a significant difference in (T2DM) versus (H) and (AP) groups for Hb1Ac. (*$P < 0.05$; ## $P < 0.01$; for both analysed parameters).

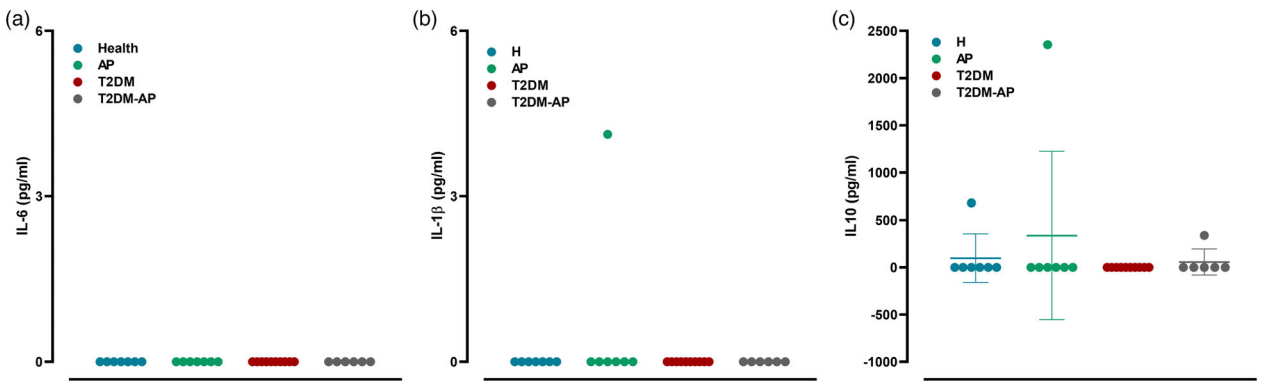

Figure 3 Serum levels of (a) IL-6, (b) IL-1β and (c) IL-10 in healthy controls (H), patients with apical periodontitis (AP), individuals with type 2 diabetes diagnosis (T2DM) or patients with both conditions (T2DM-AP), according to assessment by DuoSet ELISA assay. Results are depicted as scattered plots, showing the mean ± SD. Kruskal–Wallis test did not reveal any significant difference among the groups ($P > 0.05$).

group displayed detectable serum levels of CCL3, whilst this chemokine was found in one patient in each of the other groups (Figure 4a). Detectable serum levels of CCL4 were observed in two patients of the AP group, whereas three patients in the T2DM and T2DM-AP groups showed detectable levels of this chemokine. Alternatively, CCL4 was untraceable in serum of subjects in the H group (Figure 4b).

The Figure 5a shows that AP lesion areas were significantly higher in the T2DM-AP group, when compared with the AP group (one-way ANOVA, followed by Tukey's test; $P < 0.05$). The Figure 5b shows representative images of the AP areas in the AP (middle panel) and T2DM-AP (right panel) groups. An image of a healthy tooth apex is shown in the left panel (Figure 5b).

Discussion

The relationship between T2DM and oral health has been widely investigated. This is especially true for the link with periodontitis. However, limited evidence explored to what extent AP, which is the result of root canal infection, might influence T2DM outcomes. Herein, we present preliminary findings of an observational study

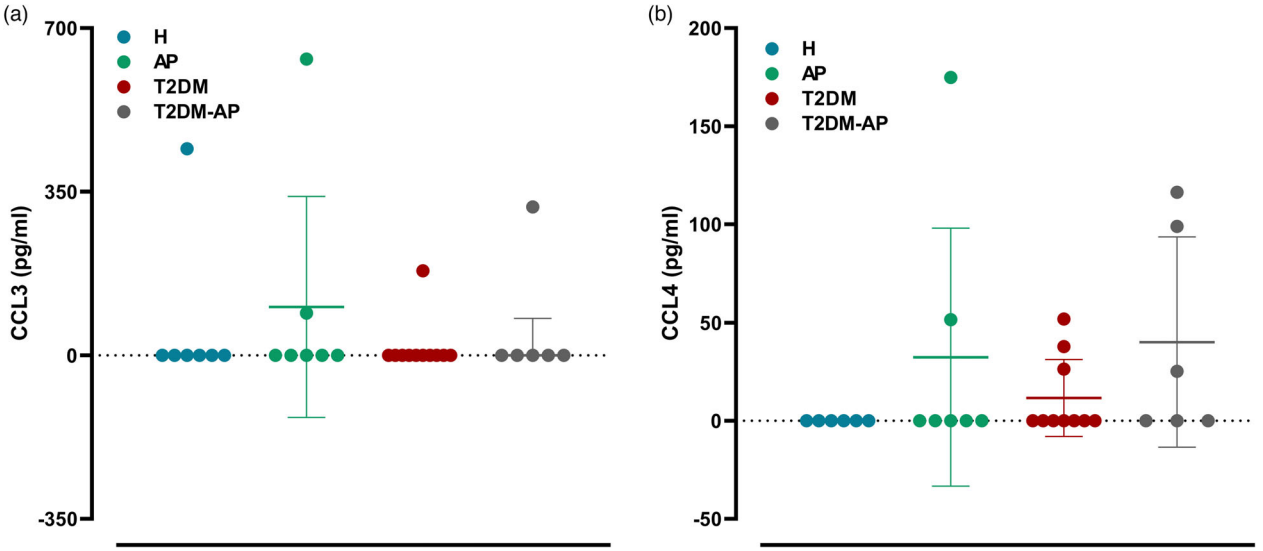

Figure 4 Serum levels of (a) CCL3, (b) CCL4 in healthy controls (H), patients with apical periodontitis (AP), individuals with type 2 diabetes diagnosis (T2DM), or patients with both conditions (T2DM-AP), according to assessment by DuoSet ELISA assay. Results are depicted as scattered plots, showing the mean ± SD. Kruskal–Wallis test did not reveal any significant difference among the groups ($P > 0.05$).

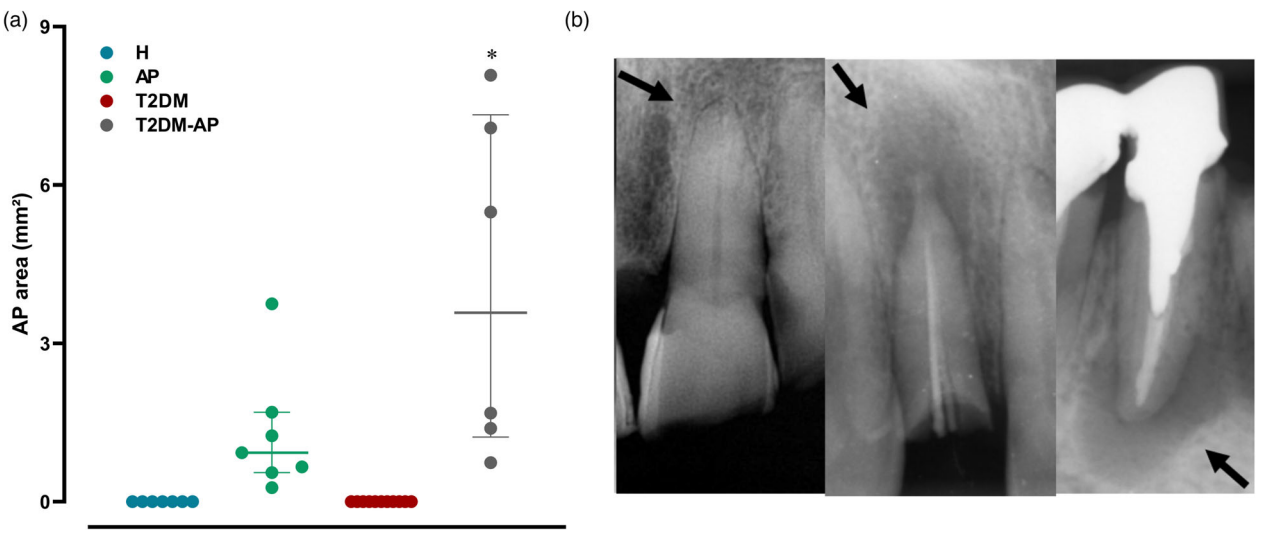

Figure 5 (a) Apical periodontitis area in healthy controls (H), patients with apical periodontitis (AP), individuals with type 2 diabetes diagnosis (T2DM), or patients with both conditions (T2DM-AP), as assessed in periapical radiographical images. Results are depicted as scattered plots, showing the mean ± SD. Ordinary one-way ANOVA reveal significant difference between AP versus T2DM-AP (*$P < 0.05$). (b) Representative images of the AP areas in the AP (middle panel) and T2DM-AP (right panel) groups. An image of a healthy tooth apex is shown in the left panel.

which suggested no influence of AP on glycaemic control or inflammatory levels amongst T2DM patients. In contrast, T2DM increased the severity of AP.

The present demographic and medical records did not show significant differences among groups, suggesting an adequate distribution of the confounding variables between case and control groups. Although the dichotomised BMI > 25 analysis did not show any significant difference, differences were found in the mean BMI of patients with T2DM, regardless of AP. Similarly, Hb1Ac levels were elevated in T2DM patients, with or without AP. In addition, the frequency of use of antihypertensive and/or lipid-lowering drugs was significantly higher in patients allocated in the T2DM or

T2DM-AP groups, which might be an indicative of diabetes-related complications in these patients, such as hypertension and dyslipidaemia. This set of results corroborates that most of the T2DM patients exhibit characteristics compatible with metabolic syndrome (21).

A poorer glycaemic control might be expected in patients with T2DM and AP when compared with the T2DM group. However, we did not find this difference in our study. All the patients with T2DM diagnosis referred to the use of metformin for diabetes treatment. Besides its effects on glycaemic control, metformin exhibits additional mechanisms of action, being able to modulate inflammatory pathways, oxidative stress and endothelial dysfunction (22). Noteworthy, previous pre-clinical studies suggested that systemic treatment or intracanal medication with metformin decreased the size of periapical lesions, by reducing the number of osteoclasts and bone resorption areas, likely via suppression of the NF-κB pathway (23,24). It is tempting to propose that metformin might inhibit the activation of inflammatory pathways secondary to root canal infection, reducing the overall effects of AP on T2DM and H1bAc results. This notion is supported by recent evidence suggesting that part of metformin benefits on T2DM are secondary to the modulation of gut microbiota towards the homeostasis state, with an alteration of more than 80 bacterial strains (25). Thus, the potential effects of metformin on AP-related bacteria might also help to explain the results of the present study. Further studies in a near future are required to investigate the microbiota in individuals with both T2DM-AP under metformin therapy.

Most literature data correlating periodontitis and T2DM propose that transient bacteraemia secondary to oral infection might sustain a systemic inflammatory response, compromising the glycaemic control (26,27). Furthermore, elevated levels of inflammatory cytokines have been associated with T2DM risk as a whole (28). AP represents an infection-related inflammatory condition that might account for the low-inflammatory grade process underlying T2DM. It was demonstrated that in the presence of AP, cytokines are detected in both the exudate of the lesion itself (29) or in serum (14). Notably, pre-clinical studies have shown that raise of serum cytokine contents is dependent on the number of AP lesions (30). In our study, the serum levels of the adipokine IL-6 were undetectable in any of the evaluated groups. Instead, literature data have suggested that increased IL-6 levels in active AP lesions might account for a systemic inflammatory response (31). A recent systematic review and meta-analysis suggested that AP leads to increased serum levels of IL-6, besides other inflammatory markers such as C-reactive protein (32). Nonetheless, one of the two studies included in this meta-analysis presented results with low levels of IL-6 (1.92 ± 1.55 pg mL^{-1}) (33). The other one showed low differences between control and AP groups (median values of 22.4 vs. 23.2) (34). Considering these considerations, the detection range of the ELISA kit used by us might explain the contrasting results–it is not as sensitive as what used in other studies. Moreover, the patients enrolled in the present study exhibited chronic AP lesions, a situation in which the levels of inflammatory mediators are expected to be reduced. Regarding IL-1β, only one patient had detectable levels of this cytokine in serum, which remain to be discussed later. A previous study that investigated the impacts of AP on cardiovascular risk factors reported an elevation of serum IL-1β levels in patients with an AP diagnosis, in comparison with control individuals (14). However, the average mean levels of IL-1β in this study did not exceed one pg mL^{-1}, which is outside the detection range of most ELISA kits, and that can be considered very low. The absence of great differences in serum IL-6 and IL-1β levels as observed by us might correlate with the modest clinical impact of chronic endodontic infection and plasma inflammatory biomarkers in hypertensive patients, as proposed by Vidal *et al.* (35).

In the present study, we have also evaluated the serum levels of IL-10, which is an anti-inflammatory cytokine essential for regulating the immune responses to microbial antigens. IL-10 is known to prevent excessive inflammation during the course of infection (36). More recently, it was demonstrated that mice lacking Toll-like receptor 2 and IL-10 develop osteomyelitis-like lesions after endodontic infection induction, further confirming a relevant role for IL-10 to control AP progression (37). Nonetheless, we had only three patients with detectable levels of IL-10, irrespective of T2DM or AP diagnosis.

Monocytes are present in the inflammatory infiltrate of AP and had been associated with the production of several inflammatory mediators (38). The CCL family of chemokines is especially important in this context, as they recruit macrophages to the inflammatory sites (39). CCL3 and CCL4 are the main chemotactic proteins produced by macrophages and adipose tissue, and enhanced tissue levels of CCL3 have been associated with obesity and metabolic inflammation (40). An analysis of chemokines in the necrotic pulp tissue did not show any difference in comparison with healthy tissues (41). Otherwise, another study reported higher levels of CCL4 in the AP lesion when compared with the controls (42). In our study, the variations of CCL3 and CCL4 contents in serum did not appear to correlate with T2DM and AP. Additional investigation is required to assess a comprehensive panel of chemokines in a large sample of T2DM patients with AP.

As discussed above, the levels of IL-1β were detectable only in one patient. The same patient displayed elevated

levels of IL-10, CCL3 and CCL4, but not IL-6. Based on the medical records, this patient had a history of prostate benign hyperplasia (BPH). It is well recognised that cytokines are implicated in the maintenance of the prostatic inflammatory process. Accordingly, elevated levels of IL-1β and CCL3 have been linked to the proliferation of fibroblasts and autocrine stromal cells in BPH (43,44). As for IL-10, in addition to this patient, two other participants had detectable levels of this cytokine: one with a BMI of 31, and one diagnosed with depression. Indeed, it has been previously demonstrated that IL-10 display a relevant role on the regulation of tryptophan metabolism in a pre-clinical model of inflammation-induced depression (45), what might support somewhat our data. All the patients with detectable levels of CCL3 and CCL4 had BMI values >25. Additionally, some patients with comorbidities, such as acute periodontal abscess, hypothyroidism or osteoporosis also had higher levels of CCL3. A patient under treatment for schizophrenia displayed enhanced serum levels of both CCL3 and CCL4. In fact, previous evidence suggested a close relationship between schizophrenia spectrum disorders and an unbalance of inflammatory mediators, including chemokines (46). Moreover, a recent study showed an increase in a series of CCL chemokines in adipose tissue of obese patients, including CCL3 and CCL4. The same cohort of obese patients also displayed higher serum levels of CCL2, CCL3 and CCL5, in comparison with non-obese individuals (47).

A prospective study suggested that AP was more prevalent in patients with T2DM and that they had larger lesions than healthy patients (48). Another publication suggested that AP areas and severe bone destruction of periapical tissues were significantly higher in patients with T2DM when compared with non-diabetic patients, as evaluated after endodontic intervention (49). Confirming and extending this notion, in our study, the AP lesion areas were significantly higher in patients with T2DM diagnosis, in relation to the respective control group. As highlighted before, the systemic treatment with metformin was able to reduce the size of periapical lesions in a rat model of AP (24). When analysed in concert with literature data, our results permit to infer that metformin might well prevent some of the impacts of AP on T2DM due to its global anti-inflammatory effects, without any effect on AP lesion extent. In this study, AP diagnosis was confirmed by two independent investigators by using the periapical index proposed by Ørstavik *et al*. (1986), which varies from 1 (absence of AP) to 5 (severe AP), according to the evaluation of periapical radiographies. Noteworthy, despite the greater AP areas in T2DM individuals, there were no significant differences regarding AP scoring in relation to healthy patients (results not shown). It has been recently suggested that treatment of T2DM patients with metformin or statins has been independently associated with a lower prevalence of AP, whereas HbA1c > 8.0 was linked to higher AP prevalence. In our study, 6 out of 10 in T2DM, and 2 out of 6 in the T2DM-AP group had HbA1c levels higher than 8.0. It is possible to infer that additional medication and/or habit changes might improve that parameter and consequently the AP extent. However, further studies in this topic are still required to address this question (50).

It is plausible to consider that usually AP represents a restrained inflammatory response, whereas periodontitis displays a spread inflammatory feature. The different infectious and inflammatory burden seen in AP and periodontitis cannot be overlooked concerning their impacts on metabolic diseases, such as T2DM. Certainly, the small sample size of this preliminary study might compromise this set of conclusions. Initially, periodontitis was an exclusion criterion; however, during the patient selection phase, it was observed that most T2DM patients presented with periodontitis. As recently reviewed, periodontitis is considered the 60 complication more related to diabetes (8). Thus, we decided to include these patients in the study regardless of periodontitis diagnosis. In a near future, we intend to evaluate periodontitis and AP, independently and associated, aiming a better understanding on how these oral infectious diseases corroborate to T2DM complications. To the best of our knowledge, there is no previous publication with a similar design of the present study, which is a limitation to further comparatively discussing our data.

Despite the limited sample size, this pilot study indicated that AP itself did not affect the glycaemic control or the levels of cytokines/chemokines in T2DM patients, even though T2DM increased the AP severity. Further studies enrolling larger samples are required to define a possible two-way liaison between AP and T2DM.

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Conflict of interest

The authors declare that they have no conflict of interest.

Author contributions

L.P.A, D.E.B., R.K.S., S.B.G.W., J.A.P., M.S.G. and M.M.C. conceived and designed research; L.P.A., D.E.B., S.B.G.W., M.S.G recruited and selected the patients;

L.P.A. collected the blood samples and performed the laboratorial analyses. L.P.A., M.S.G. and M.M.C. analysed and interpreted results of experiments; L.P.A. and M.M.C. prepared figures; L.P.A., M.S.G. and M.M.C. drafted manuscript, L.P.A, D.E.B., R.K.S., S.B.G. W., J.A.P., M.S.G. and M.M.C. revised and edited manuscript; L.P.A, D.E.B., R.K.S., S.B.G.W., J.A.P., M.S.G. and M.M.C. approved the final version of the manuscript.

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Ethical approval

All procedures involving human participants were reviewed by the Institutional Ethics Committee that approved all the protocols (report number 1.323.531).

Informed consent

Informed consent was obtained from each patient, and they were assured of the confidentiality of data.

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