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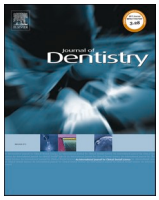

# Association between calcium-channel blockers and gingival enlargement: A case-control study

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ABSTRACT

*Objectives:* As reported by the existing literature, calcium-channel blockers (CCB) can lead to gingival enlargement. The aims of this study were to investigate the factors associated with gingival enlargement in patients on CCB and to assess the saliva and gingival crevicular fluid (GCF) profile of patients on CCB with gingival enlargement.

*Methods:* A total of 131 participants were included. Data were collected from 91 patients taking CCB for treatment of systemic hypertension. The presence of drug-induced gingival enlargement (DIGE) was assessed clinically and associated with patient factors. Patients with DIGE were group-matched for gender and ethnicity with an equal number of consecutive CCB non-DIGE patients (control 1), no-CCB no-DIGE (control 2) and periodontally healthy with no DIGE (control 3) for the saliva and GCF analysis. A bead-based multiplex immunoassay was used to assess a panel of biomarkers.

*Results:* Twenty-two percent of patients on CCB were diagnosed with DIGE. Lack of daily interdental cleaning and self-reported diagnosis of type II diabetes were associated with the diagnosis of DIGE. When analysing patients only on CCB, those with DIGE had higher GCF levels of vascular endolthelial growth factor (VEGF) ($p = 0.032$), epidermal growth factor (EGF) ($p = 0.030$) and matrix metalloproteinase-8 (MMP-8) ($p = 0.008$). Among the salivary markers, only MMP-8 showed a statistically significant difference across groups ($p < 0.001$).

*Conclusions:* This is the first study investigating saliva and GCF biomarkers in patients with DIGE and different control groups, suggesting that causes of the overgrowth might involve inflammatory processes, tissue damage pathways, and potentially an impact on growth factors like VEGF. Future research should verify these results in independent populations and explore the underlying pathogenic mechanisms in-depth.

*Clinical significance:* Calcium-channel blockers (CCB) can lead to gingival enlargement. This study confirms lack of interdental cleaning and type II diabetes as risk factors. Elevated levels of VEGF, EGF, and MMP-8 in gingival crevicular fluid and MMP-8 in saliva suggest inflammatory processes and growth factors might play roles in this condition.

## 1. Introduction

Drug-induced gingival enlargement (DIGE) can be described as an increase in gingival tissue volume after the regular use of specific systemic drugs such as calcium-channel blockers (CCBs), the antivonvulsant Phenytoin and the immunosuppressasnt Ciclosporin [1]. CCBs in particular represent one of the first line for the treatment of hypertension and are still the most commonly prescribed drugs in hypertensive patients [2]. The pathogenetic mechanism of drug-induced gingival overgrowth follows a multifactorial model, and the histological features and clinical correlation between CCBs and gingival enlargement have been described by different research groups [3]. However, few studies conducted standardised *in-vitro* or animal models experiments with conclusive results about the etiology of CCBs-related gingival enlargement [1]. It is unclear to what extent different risk factors such as dental plaque, age, genetic factors, drug dosage and duration are involved in the onset of DIGE [4]. Moreover, the prevalence of DIGE in patients using CCBs is still uncertain [5], ranging from 3.4%

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[6] to 76% [2] when amlodipine was prescribed to manage hypertension.

Two mechanisms have been proposed to explain the DIGE phenomenon associated with CCBs: the non-inflammatory process [7] and the inflammatory process [8]. It seems that through both processes, different growth factors are upregulated, resulting in an increased size of gingival tissues and inflammation [7,8]. This is particularly relevant in cases of co-existing periodontitis [9] and in the presence of subgingival plaque[10], leading to upregulation of several inflammatory markers that may contribute to the gingival enlargement. In fact, an increased CCB concentration was directly found in the gingival crevicular fluid (GCF) of patients with DIGE [4]. Previous reports showed how the inflammatory markers detected in GCF from patients with periodontitis may give an overview on the general inflammatory state following the destruction of periodontal supportive tissues [11].

Previous cross-sectional analysis of GCF samples from periodontitis patients taking amlodipine assessed the levels of Interleukin-17A (IL-17A) [12], transforming growth factor-b1 (TGF-b1), platelet-derived growth factor-BB (PDGF-BB), and basic fibroblast growth factor (bFGF) [13]. Few studies evaluated saliva samples from patients taking CCBs, and they reported that CCBs might cause dry mouth by inhibiting saliva secretion [14]. A study also reported that there was no difference in the nitrite and nitrate concentrations between patients taking nifedipine and non-nifedipine patients [15]. However, larger studies with control groups are needed to investigate saliva and GCF biomarkers in patients with DIGE. Therefore, the aims of this case-control study were to 1) assess factors associated with DIGE in patients on CCB, and 2) to assess the saliva and GCF profile of patients on CCB with DIGE, comparing them with those of patients with untreated periodontitis with no DIGE, and to patients not on CCB and without DIGE (with periodontitis). The null hypotheses were that no saliva or GCF biomarkers would differentiate between CCB patients with DIGE from CCB non-DIGE patients, and non- CCB periodontitis patients.

## 2. Materials and methods

The study was conducted in two parts. Part one consisted of a cross-sectional study of consecutive patients on CCB, investigating factors and demographics associated with gingival enlargement and part two was a case-control study investigating the GCF and salivary biomarker profiles of CCB patients with DIGE and matched controls. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines were followed, available in the Supplementary file section.

### 2.1. Patient population

Samples were collected from patients taking part in the King's College London Oral, Dental and Craniofacial Biobank. The Biobank was granted ethics approval by the East of England-Cambridge East Research Ethics Committee (reference 20/EE/0241). The study was conducted according to the principles of the Declaration of Helsinki. The privacy rights of human subjects have been observed and each patient gave written consent to take part in the Biobank recruitment between December 2020 and June 2023 in the Periodontology new patients' clinic at the Guy's & St Thomas Foundation Trust Hospital (GSTFT). Specific approval for the release of data and samples for this study was granted by the Biobank Management Committee (Biobank reference 009). Among 848 patients who had consented to the Biobank up to 26th June 2023, all patients taking calcium-channel blockers (CCB) for treatment of hypertension for at least 12 months were included in the analysis. Out of these, all patients diagnosed with DIGE (see definition below) were group-matched for gender and ethnicity with an equal number of consecutive CCB non-DIGE patients (control 1), no-CCB no-DIGE (control 2) and periodontally healthy with no DIGE (control 3) for the saliva and GCF analysis.

### 2.2. Clinical examination

Following consent, demographic and medical parameters were collected, along with dental history. Self-reported smoking habit was recorded (number of cigarettes/days, years of smoking). Medical history included questions about calcium-channel blockers (type of medication, dose and number of years of taking it). Height, weight and waist measurements were taken at the study visit. The following periodontal measurements were taken at six sites/tooth by Biobank examiners using a UNC-15 periodontal probe: dichotomous full mouth plaque scores (FMPS) [16], full mouth probing pocket depth (PPD), recession (REC) of the gingival margin from the cemento-enamel junction (CEJ), bleeding on probing (BOP) [17], tooth mobility [18] and furcation involvement [19]. Clinical attachment level (CAL) was calculated as PPD+REC.

### 2.3. Periodontal diagnosis

Periodontal diagnosis was based on the current classification of periodontal diseases [20], using the following criteria:

- Periodontal health: BOP $<$10% and PPD $\leq$4 mm and no site $\geq$4 mm PPD with BOP with evidence of previous bone loss/attachment loss
- Gingivitis: BOP $\geq$10% with all sites with PPD $\leq$3 mm
- Periodontitis: presence of $\geq$2 non-adjacent sites with PD 4 mm and BOP or $>$4 mm PPD with evidence of radiographic bone loss (except in case of deep caries, endo-periodontal pathology, fracture, third molars) [21]. Patients with periodontitis were further subdivided according to staging and grading [20].

Diagnosis of DIGE was given when the size of the gingival unit was greater than would normally be expected from purely an inflammatory reaction in the gingival tissues [22]. Therefore, a diagnosis of DIGE was recorded if the gingival margin was at least 2 mm coronal to the CEJ. According to the current periodontitis classification, the extent of gingival enlargement was defined as either localised or generalised. Mild gingival enlargement involves enlargement of the gingival papilla; moderate gingival enlargement involves enlargement of the gingival papilla and marginal gingiva, and severe gingival enlargement involves enlargement of the gingival papilla, gingival margin, and attached gingiva [22,23]. Although different Biobank examiners carried out patient examinations, the periodontal diagnosis was checked and confirmed by a single experienced investigator (author LN).

### 2.4. Saliva and gingival crevicular fluid collection and volume determination

A total of 5 ml of unstimulated saliva was obtained by passive drooling [24]. After rinsing with water, participants were instructed to let the saliva pool in the floor of the mouth and to then expectorate into a sterile tube for up to 10 min.

Gingival crevicular fluid (GCF) was collected from the mesial sulcus of all first molars using Periopaper (PerioPaper Strips, OraFlow Inc., NY, USA). GCF sampling was performed prior to periodontal probing to avoid blood contamination. After isolation the teeth with cotton rolls and gentle drying, GCF was collected by placing the Periopaper into the gingival sulcus until mild resistance was felt and then leaving it for 30 s. Samples visually contaminated with blood or diluted with saliva during sampling were discarded.

The GCF volume was estimated chair side using the Periotron device (Periotron 8000, OraFlow Inc., NY, USA) with the volume expressed in Periotron units. The Periotron device was calibrated according to the manufacturer's instructions and with a sterile unused Periopaper before each measurement. To extrapolate the GCF volume from the Periotron units, the formula $y = a + bx^c$ was used, where y is the periotron score in units, x is the volume in μl, a is the intercept and is 0, b is 135 for serum, and c is 0.834.

The strip was stored in an Eppendorf tube and transferred to −80 °C. GCF was eluted using PBS with protease inhibitors 1X (1 tablet for 10 ml of PBS) (Complete ULTRA tablets, Mini; EDTA-free). Briefly, 50 μl of PBS/protease inhibitor cocktail was added and centrifuged at 11,000 g for 15 min at 4 °C. An additional 50 μl of PBS/protease inhibitor cocktail was added and centrifuged at 11 000 g for 15 minutes at 4 °C to a total volume of 100 μl. The eluted GCF was stored at −80 °C until analysis.

### 2.5. GCF and saliva biomarkers analysis

The following biomarkers were investigated in both GCF and saliva sample: epidermal growth factor (EGF), vascular endothelial growth factor (VEGF), transforming growth factor beta (TGF-)β, acidic fibroblast growth factor (FGFa), basic fibroblast growth factor (FGFb), interleukin 1 alpha (IL-1α), interleukin 6 (IL-6) and matrix metalloproteinase-8 (MMP-8).

The analysis of was carried out using a bead-based multiplex immunoassay, following the manufacturer's instructions (Luminex; R&D systems). Saliva samples were centrifuged before dilution to eliminate debris and run the analysis smoothly. Each sample was analysed in duplicates. Briefly, samples were eluted with a calibrator diluent, mixed with a microparticle cocktail, and incubated for 2 h. Following three cycles of washes to remove any unbound microparticle cocktail, a biotinylated antibody cocktail (Biotin) was added and incubated for 1 hour. Another three cycles of washes were performed before adding streptavidin phycoerythrin (Streptavidin-PE) and incubating for 30 min. The Biotin served as a binding site for Streptavin, allowing analytes to be detected. Finally, several washes were performed, followed by adding 100μl wash buffer and incubating for two minutes before reading the plate using a Luminex MAGPIX analyser (Luminex; R&D systems), and the concentrations for each marker were reported in pg/ml.

### 2.6. Statistical analysis and sample size calculation

Data from all included patients were entered into a spreadsheet and proofread for entry errors. Data analysis was performed using SPSS for Windows version 27.0 (IBM Corp., Armonk, NY).

The primary outcome was VEGF levels between the 4 study groups ($n = 20$ each). As no studies were available reporting GCF analysis in DIGE vs non-DIGE using the selected biomarkers, differences in VEGF in periodontal defects vs. healthy sites, supposing a difference of 85 pg/ml (SD 170) [25] were used, resulting in 70 cases needed to provide 90% power. Therefore, the selected sample of $n = 80$ has more than 90% power and can compensate for the loss of some samples during analysis.

An initial analysis was carried out to detect associations between patient factors and the presence of DIGE in the overall sample of patients on CCB, by Chi-square or ANOVA for categorical and continuous variables respectively. Logistic regression was then carried out, with DIGE as outcome, and age, gender, ethnicity, smoking, BMI, diabetes, dose and duration of CCB and interdental cleaning as explanatory variables. Following this, the associations between the 4 study groups ($n = 20$ each), GCF volume, and biomarker levels in saliva and GCF were investigated by non-parametric approaches (Mann-Whitney and Kruskal-Wallis depending on the number of groups tested) as biomarker data were not normally distributed. A $p$ value $< 0.05$ was considered statistically significant. Bonferroni corrections were used for the biomarker analyses, due to the multiple comparisons.

## 3. Results

### 3.1. Patient population

Table 1 reports the demographic and clinical characteristics of all patients included in the study. A total of 91 patients were identified as taking CCB for at least 12 months. Out of these patients, 20 (22%) were diagnosed with DIGE, while 71 (78%) were found not to have DIGE. Most patients were found to have periodontitis, with only one DIGE patient was diagnosed with gingivitis, one non-DIGE diagnosed with periodontal health and 12 non-DIGE diagnosed with gingivitis. DIGE patients showed a mean hemoglobin glycosylated A1c (HbA1c) of 6.6 ± 2.1%, whereas non-DIGE patients had a mean HbA1c of 5.8 ± 1.3% ($p < 0.057$). All diabetic patients reported to have controlled diabetes, although 4 of them had HbA1c values > 7%.

**Table 1**
Characteristics of patients on calcium-channel blockers (CCB), divided based on diagnosis of drug-induced gingival overgrowth (DIGE). Mean ± standard deviation is reported for continuous variables. The comparison between groups was calculated by Chi-square (categorical variables) or ANOVA (continuous variables).

| | | Frequency | Mean ± SD | Frequency | Mean ± SD | Comparison p= |
|---|---|---|---|---|---|---|
| | | DIGE ($n = 20$) | | Non-DIGE ($n = 71$) | | |
| **Age** | | | 59.8 ± 10.3 | | 60.3 ± 10.5 | 0.836 |
| **BMI** | | | 28.8 ± 5.1 | | 31.0 ± 10.0 | 0.397 |
| **Gender** | **Male** | 10 (50%) | – | 34 (47.9%) | – | 0.534 |
| | **Female** | 10 (50%) | – | 37 (52.1%) | – | |
| **Ethnicity** | **Caucasian** | 4 (20%) | – | 31 (43.7%) | – | 0.070 |
| | **Afro-Caribbean** | 12 (60%) | – | 29 (40.8%) | – | |
| | **Other/mixed** | 4 (20%) | – | 11 (15.5%) | – | |
| **Smoking** | **Never** | 11 (55%) | | 44 (62%) | | 0.831 |
| | **Former** | 7 (35%) | | 20 (28.2%) | | |
| | **Current** | 2 (10%) | | 7 (9.9%) | | |
| **Diabetes type II** | **Yes** | 11 (55%) | | 21 (18.9%) | | 0.001 |
| | **No** | 9 (45%) | | 90 (81.1%) | | |
| **CCB type** | **Amlodipine** | 19 (95%) | – | 63 (88.7%) | – | 0.367 |
| | **Nifedipine** | 2 (10%) | – | 6 (8.4%) | – | |
| | **Felodipine** | 0 | – | 2 (2.8%) | – | |
| **CCB dose** | **≤ 5mg** | 7 | – | 25 | – | 1.000 |
| | **10mg** | 18 | – | 36 | – | |
| | **unknown** | 2 | – | 10 | – | |
| **CCB duration (years)** | | | 10.3 ± 7.7 | | 6.6 ± 5.2 | 0.033 |
| **Brushing frequency** | **<2/day** | 2 (10%) | | 15 (13.7%) | | 1.000 |
| | **At least 2/day** | 18 (90%) | | 93 (86.3%) | | |
| **Daily interdental cleaning** | **Yes** | 10 (50%) | | 69 (63.3%) | | 0.320 |
| | **no** | 10 (50%) | | 40 (36.7%) | | |
| **Periodontal diagnosis** | **Health** | 0 | – | 1 (1.4%) | – | 0.321 |
| | **Gingivitis** | 1 (5%) | – | 12 (17.4%) | – | |
| | **Periodontitis** | 19 (95%) | – | 56 (81.2%) | – | |

### 3.2. Associations between patient factors and DIGE in patients on CCB

Univariate associations between patient factors and DIGE are reported in Table 1. Only the presence of type II diabetes ($p = 0.001$) and longer duration of CCB use ($p = 0.033$) were statistically significantly associated with a higher incidence of DIGE. Logistic regression revealed that both lack of daily interdental cleaning ($p = 0.022$, OR=9.64, 95% CI= 1.38–67.24) and self-reported diagnosis of type II diabetes ($p = 0.024$, OR=8.73, 95% CI=1.33–57.08) were associated with the diagnosis of DIGE.

### 3.3. GCF analysis

For the GCF analysis, the 20 DIGE patients were group-matched for gender and ethnicity with 20 CCB non-DIGE (control 1, consecutive matched cases from the 71 non-DIGE described above), 20 non-CCB periodontitis (control 2) and 20 non-CCB periodontally healthy (control 3) cases (see Fig. 1). Table 2 reports the characteristics of these 80 patients. Periodontally healthy patients were, on average, younger ($p < 0.001$) and had a smaller BMI ($p < 0.001$) compared with the other three groups. A higher number of diabetic patients was found in the test group ($p < 0.001$). As estimated by the Periotron average GCF volume was slightly lower in healthy subjects, but no statistically significant differences were detected across the four groups.

Table 3 shows results of GCF biomarker analysis for participants divided by study group. When analysing only patients on CCB ($n = 40$), those with DIGE ($n = 20$) had almost three times higher levels of MMP-8 ($p = 0.008$), more than double VEGF ($p = 0.032$) and increased EGF ($p = 0.030$) compared with patients with no DIGE. EGF, VEGF and MMP-8 all showed statistically significant differences when comparing across the four groups, as well as for CCB vs. periodontitis non-CCB. IL-6 levels were higher in the CCB groups (with and without DIGE) compared to periodontitis patients not on CCB ($p = 0.024$).

### 3.4. Saliva analysis

Table 4 shows the results of saliva biomarker analysis for participants divided by study group. CCB patients with DIGE tended to have higher salivary levels of most biomarkers. However, statistically significant differences across groups were detected only for MMP-8 ($p < 0.001$). No statistically significant differences were detected when comparing CCB DIGE vs. CCB non-DIGE and when comparing CCB cases with non-CCB periodontitis cases.

Among studied biomarkers, statistically significant correlations were detected between GCF and saliva only for MMP-8 (0.019 at Paerson correlation), while the correlation was border-line for FGF acidic ($p = 0.055$).

## 4. Discussion

This study showed that more than a fifth of patients on CCB were affected by DIGE. Lack of daily interdental cleaning and self-reported diagnosis of type II diabetes were associated with the diagnosis of DIGE. All patients reported to have controlled diabetes, although four of them had HbA1c > 7%. Interesting data emerged from the saliva and GCF bead-based multiplex immunoassays, which may shed some light in the yet-not-clearly understood pathogenesis of DIGE.

The overall prevalence of DIGE (22%) in the present study is in line with previous studies [2,6]. However, this has to be interpreted cautiously, as it relates to prevalence in a non-random set of patients referred for treatment. Furthermore, there is some heterogeneity in the literature in terms of both the prevalence and incidence of DIGE [5,26]. Remarkably, as summarised by a recent review, most of the recent case reports found severe DIGE in patients taking 5–10 mg of CCBs daily [5], even only after 3 months of amlodipine intake [27].

The association between diabetes and DIGE has long been suggested [28]. Case reports showed that simultaneous presence of uncontrolled diabetes and intake of amlodipine, felodipine and nifedipine led to DIGE [29]. However, stronger evidence to confirm this synergetic association and the exact role of the hyperglycaemia is still lacking. The association between lack of interdental cleaning and DIGE is also intuitive and consistent with previous literature, as it appears that the presence of plaque seems to increase the extent of DIGE, irrespective of the initiating drug [10]. Case reports showed that meticulous plaque control (both self-administered and professionally delivered) might reduce nifedipine-induced GE [30]. A recent cross-sectional study on 162 Brazilian individuals taking 3 different CCBs (nifedipine, amlodipine and felodipine) reported that poor oral hygiene (elevated plaque index) was

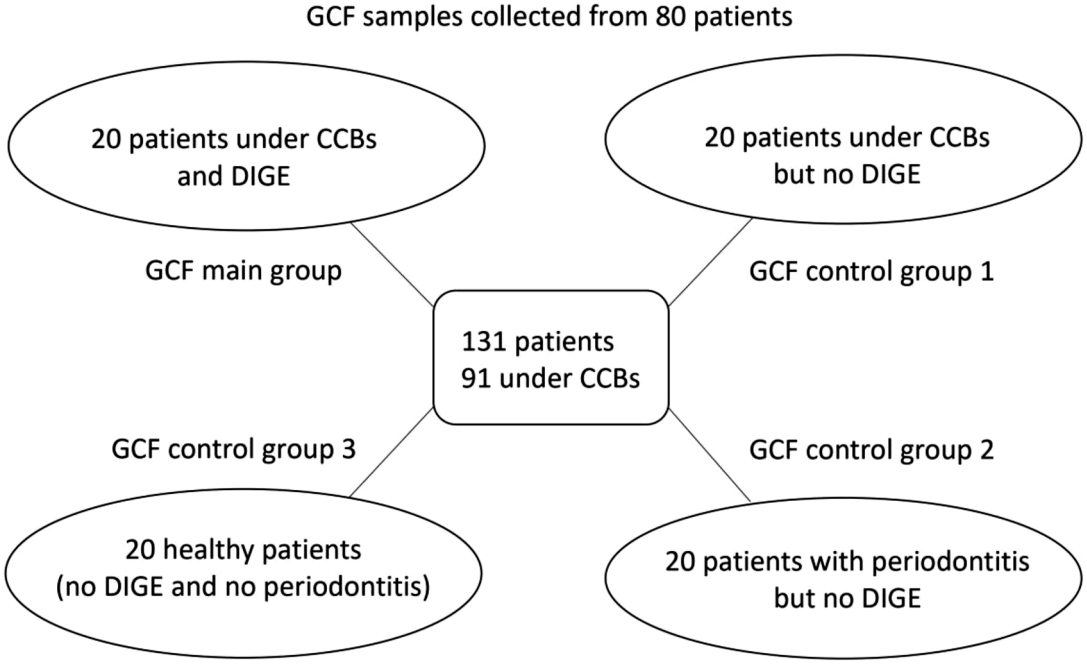


**Fig. 1.** Illustration of patients and assigned groups.

**Table 2**
Characteristics of patients included in GCF analysis. The comparison between groups was calculated by Chi-square (categorical variables) or ANOVA (continuous variables).

| | | DIGE ($n = 20$) | CCB non-DIGE ($n = 20$) | Non-CCB periodontitis ($n = 20$) | Non-CCB healthy ($n = 20$) | Comparison p= |
|---|---|---|---|---|---|---|
| **Age (mean ± SD)** | | 59.8 ± 10.3 | 57.8 ± 7.5 | 47.2 ± 11.9 | 37.0 ± 14.1 | <0.001 |
| **BMI (mean ± SD)** | | 28.8 ± 5.1 | 30.3 ± 6.5 | 30.1 ± 4.7 | 23.4 ± 3.4 | <0.001 |
| **Gender** | **Male** | 10 (50%) | 10 (50%) | 10 (50%) | 10 (50%) | 1.000 |
| | **Female** | 10 (50%) | 10 (50%) | 10 (50%) | 10 (50%) | |
| **Ethnicity** | **Caucasian** | 4 (20%) | 4 (20%) | 4 (20%) | 4 (20%) | 1.000 |
| | **Afro-Caribbean** | 12 (60%) | 12 (60%) | 12 (60%) | 12 (60%) | |
| | **Other/mixed** | 4 (20%) | 4 (20%) | 4 (20%) | 4 (20%) | |
| **Smoking** | **Never** | 11 (55%) | 10 (50%) | 12 (60%) | 17 (85%) | 0.110 |
| | **Former** | 7 (35%) | 6 (30%) | 3 (15%) | 3 (15%) | |
| | **Current** | 2 (10%) | 4 (20%) | 5 (25%) | 0 | |
| **Diabetes type II** | **Yes** | 11 (55%) | 3 (15%) | 2 (10%) | 1 (5%) | <0.001 |
| | **No** | 9 (45%) | 17 (85%) | 18 (90%) | 19 (95%) | |
| **Periodontal diagnosis** | **Health** | 0 | 0 | 0 | 20 (100%) | <0.001 |
| | **Gingivitis** | 1 (5%) | 1 (5%) | 0 | 0 | |
| | **Periodontitis** | 19 (95%) | 19 (95%) | 20 (100%) | 0 | |
| **Periotron reading (average from 4 sites)** | | 71.9 ± 29.1 | 85.5 ± 35.1 | 78.7 ± 41.2 | 62.6 ± 31.8 | 0.224 |

**Table 3**
Mean and standard deviations of GCF biomarkers in the four study groups. Comparisons were carried out by non-parametric tests.

| | DIGE ($n = 20$) | CCB non-DIGE ($n = 20$) | Non-CCB periodontitis ($n = 20$) | Non-CCB healthy ($n = 20$) | Comparison between CCB-DIGE and CCB non-DIGE p= | Comparison between CCB vs non-CCB periodontitis | Comparison across 4 groups n= |
|---|---|---|---|---|---|---|---|
| **EGF (pg/ml)** | 18.3 ± 12.7 | 10.0 ± 8.5 | 9.2 ± 12.0 | 16.7 ± 18.3 | 0.030[a] | 0.040[a] | 0.016[a] |
| **VEGF (pg/ml)** | 104.6 ± 88.0 | 45.8 ± 42.1 | 31.5 ± 27.2 | 50.3 ± 30.7 | 0.032[a] | 0.006[q] | <0.001[a] |
| **FGF acidic (pg/ml)** | 39.3 ± 64.4 | 26.9 ± 51.7 | 15.0 ± 20.9 | 40.2 ± 38.4 | 0.816 | 1.000 | 0.272 |
| **FGF basic (pg/ml)** | 0.6 ± 0.6 | 1.0 ± 2.5 | 0.9 ± 1.5 | 0.3 ± 0.2 | 1.000 | 1.000 | 1.000 |
| **IL-1 alpha (pg/ml)** | 490.0 ± 287.9 | 310.7 ± 189.7 | 347.9 ± 277.8 | 554.9 ± 692.6 | 0.240 | 1.000 | 1.000 |
| **IL-6 (pg/ml)** | 6.5 ± 4.5 | 6.3 ± 9.1 | 3.3 ± 3.7 | 3.6 ± 2.5 | 1.000 | 0.024[a] | 0.064 |
| **IL-17 (pg/ml)** | 1.6 ± 0.9 | 1.6 ± 1.0 | 1.4 ± 1.4 | 2.0 ± 1.2 | 1.000 | 0.648 | 0.704 |
| **MMP-8 (pg/ml)** | 110,760.4 ± 106,314.9 | 38,708.8 ± 43,632.3 | 40,251.6 ± 88,271.2 | 35,274.4 ± 37,082.4 | 0.008[a] | 0.008[a] | 0.002[a] |

[a] Statistically significant difference.

**Table 4**
Mean and standard deviations of saliva biomarkers in the four study groups. Comparisons were carried out by non-parametric tests.

| | DIGE ($n = 20$) | CCB non-DIGE ($n = 20$) | Non-CCB periodontitis ($n = 20$) | Non-CCB healthy ($n = 20$) | Comparison between CCB-DIGE and CCB non-DIGE p= | Comparison between CCB vs non-CCB periodontitis | Comparison across 4 groups n= |
|---|---|---|---|---|---|---|---|
| **EGF (pg/ml)** | 1084.37 ± 683.79 | 1020.62 ± 522.90 | 676.92 ± 401.57 | 730.28 ± 513.57 | 1.000 | 0.121 | 0.224 |
| **VEGF (pg/ml)** | 3249. 48 ± 1604.18 | 2822.58 ± 1883.17 | 2734.20 ± 2179.48 | 2335.73 ± 1123.52 | 1.000 | 1.000 | 1.000 |
| **FGF acidic (pg/ml)** | 26.98 ± 34.23 | 23.88 ± 14.34 | 25.41 ± 12.05 | 17.82 ± 12.46 | 1.000 | 1.000 | 1.000 |
| **FGF basic (pg/ml)** | 3.07 ± 5.15 | 3.40 ± 6.51 | 1.96 ± 1.70 | 1.03 ± 1.35 | 1.000 | 1.000 | 1.000 |
| **IL-1 alpha (pg/ml)** | 876.69 ± 1235.31 | 482.42 ± 334.29 | 372.15 ± 301.48 | 308.71 ± 198.19 | 0.856 | 0.248 | 0.056 |
| **IL-6 (pg/ml)** | 101.65 ± 69.83 | 103.91 ± 57.98 | 94.67 ± 37.14 | 114.44 ± 52.24 | 1.000 | 1.000 | 1.000 |
| **IL-17 (pg/ml)** | 21.57 ± 67.23 | 13.55 ± 24.93 | 6.37 ± 3.40 | 8.45 ± 14.63 | 1.000 | 1.000 | 1.000 |
| **MMP-8 (pg/ml)** | 98,424.36 ± 29,104.49 | 81,091.34 ± 36,558.90 | 72,562.84 ± 35,685.37 | 35,276.35 ± 17,542.92 | 0.592 | 0.640 | <0.001* |

* Statistically significant difference.

significantly associated with GE [31]. However, some authors considered plaque accumulation not an essential factor that resulting in GE [32].

No statistically significant association was detected between dose and duration of CCB and DIGE in the present study, although DIGE patients had been on CCB for an average 10.3 years, compared with 6.6

years for those with no DIGE. The relationship between dose/duration of CCBs intake and DIGE is controversial [33]. A randomised controlled trial on 19 patients with cardiovascular diseases on nifedipine observed that drug dosage was not correlated to DIGE changes [34]. In general, no significant differences in DIGE severity was noted with different doses of CCBs, although a decrease can appear after dose reduction [35]. Overall, DIGE can occur in individuals taking any amount of CCBs [5].

Bead-based multiplex immunoassays from the saliva and GCF of DIGE patients and three groups of matched controls revealed some interesting findings. Although no differences in GCF volume (as measured by the Periotron) were detected, some of the investigated markers, namely EGF, VEGF and MMP-8, were particularly increased in DIGE patients. When only assessing patients taking CCB, those with DIGE had higher GCF levels of EGF, VEGF and MMP-8. The increase in matrix metalloproteinases is consistent with what is observed in patients with periodontal destruction [36]. Furthermore, treatment with nifedipine has been suggested to increase MMP-9 gene and protein expression levels in the presence of local inflammation [37]. MMP-8 and MMP-9 expression levels were found to be higher in patients with DIGE and distinguished moderate from mild DIGE in a recent study [38]. This may be mediated by a higher proliferation, migration abilities and increased transcriptional gene expression of fibroblasts [39]. A previous in vitro study also found that amlodipine can increase the proteolytic activity of MMP-2 and inhibit the transcription of tissue inhibitor of metalloproteinase-2 (TIMP-2) in both fibroblasts and vascular smooth muscle cells [40]. A plausible regulatory mechanism of MMP-2 activation might be related to changes in intracellular calcium concentrations, although which MMPs are mostly affected is still unclear [41]. An increase in IL-1 beta GCF levels in DIGE patients is in line with previous studies [42], but it was not statistically significant after Bonferroni corrections.

The increase in VEGF is probably an essential mechanism integral to the increase vascular proliferation in gingival environment. The effect of CCB on VEGF is supported by an in vitro study showing that nifedipine stimulated VEGF production from human coronary artery smooth muscle cells (HCSMCs) through activation of protein kinase C (PKC) inhibitors via the bradykinin B2 receptor antagonist [43]. Very recently, Kumar and co-workers found that amlodipine therapy increased VEGF both in vitro and in vivo [44]. However, these effects have not always consistently been found, possibly depending on CCB dosage and study experimental design [45]. Interestingly, other medications that contribute to DIGE such as phenytoin and cyclosporin have demonstrated elevations in levels of VEGF [46]. Furthermore, fibroblasts from patients on nifedipine have been shown to have an increased number of EGF receptors compared to non-drug-treated control [47]. This increase in EGF and VEGF is probably secondary or parallel to increases in inflammatory cytokines such as IL-1 in GCF, and part of a concerted inflammation and proliferation/repair phase in the periodontal tissues [11,25].

The present study also showed that patients on CCB had higher GCF levels of IL-6 than those not on CCB. This seems to suggest that IL-6 increases are associated with the use of CCB but not with the process of DIGE. An in vitro study on primary human lung vascular smooth muscle cells (VSMC) observed that amlodipine, diltiazem and verapamil had a stimulatory effect on transcriptional activity of the human IL-6 promoter-luciferase construct pIL6-luc651, leading to a direct activation of the IL-6 gene transcription via factors NF-IL6 and NF-kB, and thus, up-regulating IL-6 mRNA and protein levels [48]. Another in vitro study observed a significant increase in IL-6 levels after 3 weeks of amlodipine administration and acute LPS stimulation [49].

A similar trend of increase in examined inflammatory and growth factor biomarkers was observed in the saliva of patients on CCB, particularly those with DIGE, although only MMP-8 levels reached statistically significant differences across groups, mirroring the results relative to MMP-8 observed in GCF. Compared with the GCF data, fewer salivary biomarkers were increased in DIGE cases, suggesting a more marked elevation of biomarkers at the local level (GCF) rather than in saliva. However, no meaningful comparison could be made with the existing literature, due to the paucity of studies on salivary levels of the aforementioned biomarkers.

The strength of this study is the novelty of analysis of multiple saliva and GCF markers in patients with DIGE, in comparison with 3 different control groups. In addition, this is the first case-control study that evaluated salivary biomarkers from patients taking CCBs. Indeed, there are limitations associated with this study, such as the relatively small sample size, the absence of some covariates such as plaque score and the fact that GCF taken from index teeth were not from specific teeth with DIGE. However, most of the sampled sites exhibited gingival enlargement.

## 5. Conclusions

In conclusion, this study suggests that a reasonably significant proportion of patients develop DIGE within a few years of taking daily calcium-channel blockers. The mechanisms leading to gingival enlargement include inflammatory and tissue destruction pathways, but growth factors such as EGF and VEGF may play an important role. As previously reported in the literature, co-existence of diabetes and lack of interdental cleaning may amplify the inflammatory process in the periodontium, by activating the inflammatory-vascular repair pathways resulting in gingival enlargement. Therefore, it is imperative that they are controlled in patients on CCB, in order to prevent further gingival enlargement. Future studies should confirm these findings in independent populations and further investigate pathogenic mechanisms.

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## CRediT authorship contribution statement

**Giuseppe Mainas:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Pasquale Santamaria:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Noha Zoheir:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Meaad Mohammed Alamri:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Francis Hughes:** Writing – review & editing, Methodology, Formal analysis. **Emily Ming-Chieh Lu:** Writing – review & editing, Methodology, Investigation, Formal analysis. **Luigi Nibali:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization.

## Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Luigi Nibali reports equipment, drugs, or supplies was provided by Bio-Techne Corporation. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Giuseppe Mainas, Pasquale Santamaria, Noha Zoheir and Meaad Alamri contributed to conception, design, data acquisition, analysis, and interpretation, drafted and critically revised the manuscript. Emily Lu and Francis Hughes contributed to analysis and interpretation, and critically revised the manuscript. Luigi Nibali contributed to conception, design, data analysis and interpretation, drafted and critically revised the manuscript. All authors gave final approval and agree to be accountable for all aspects of the work. Authors Giuseppe Mainas, Pasquale Santamaria, Noha Zoheir and Meaad Alamri equally contributed to this work. This work was facilitated by the Dental, Oral and Craniofacial Biobank and Oral Clinical Research Unit, at the Faculty of Dental Oral Craniofacial Sciences, King's College London (REC reference: 20/EE/0241).

## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jdent.2024.105315.

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