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Nuclear Abnormalities and Cytomorphometric Characteristics of Buccal Epithelial Cells Across Glycemic Categories and Periodontitis Stages: A Cross-Sectional Study

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Abstract

This study compared nuclear abnormalities and cytomorphometric characteristics of exfoliated buccal epithelial cells across clinical groups defined by glycemic status and periodontal stage. A total of 156 participants were allocated into seven groups based on glycemic status and periodontal condition: four groups with type 2 diabetes stratified by HbA1c level (<7% or ≥7%) and periodontitis stage (Stage I–II or Stage III–IV), two periodontitis groups without diabetes (Stage I–II or Stage III–IV), and a periodontally healthy reference group without diabetes. Exfoliated buccal cells were assessed for micronuclei, binucleated cells, perinuclear halos, inflammatory cell presence, nuclear area (NA), cytoplasmic area (CA), and nuclear-to-cytoplasmic (N/C) ratio. Data were analyzed using multivariable regression models adjusted for age group and sex. Significant adjusted differences were observed across the seven clinical groups for micronuclei, binucleated cells, inflammatory cell presence, NA, CA, and the N/C ratio. The highest micronucleus and binucleated cell counts were generally observed in the HbA1c ≥ 7% groups ($p < 0.001$). NA and the N/C ratio were higher in groups with diabetes than in groups without diabetes, with the highest values observed in the HbA1c ≥ 7% groups. Diabetes duration showed no consistent association with cytogenetic or morphometric parameters. Within the clinical groups examined, differences in buccal epithelial nuclear abnormalities and cytomorphometric measures were generally more consistent across glycemic categories than between periodontal stages within the same glycemic category.

Keywords: diabetes mellitus; glycated hemoglobin; micronucleus; periodontitis; exfoliative cytology

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1. Introduction

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by persistent hyperglycemia and is associated with a wide range of systemic complications affecting the cardiovascular, renal, ocular, and nervous systems. Globally, the burden of diabetes continues to rise, with an estimated 529 million individuals affected in 2021, corresponding to a global prevalence of 6.1% [1,2]. Beyond its metabolic consequences, diabetes has been increasingly recognized as a condition that influences inflammatory responses and tissue

integrity at multiple levels. Glycemic control, commonly assessed using hemoglobin A1c (HbA1c), plays a central role in determining the severity of diabetes-related complications and reflects long-term metabolic status [3].

Periodontitis is a chronic, biofilm-associated inflammatory disease characterized by gingival inflammation, periodontal pocket formation, clinical attachment loss, and alveolar bone destruction [4]. In 2021, more than 1 billion people worldwide were affected by severe periodontitis, corresponding to a global age-standardized prevalence of 12.5% [5]. DM and periodontitis are two highly prevalent chronic diseases with a well-established bidirectional relationship. While periodontitis may adversely affect glycemic control and accelerate the development of diabetic complications, diabetes is recognized as a major risk factor for the initiation and progression of periodontal disease. Higher HbA1c levels have been shown to be associated with increased periodontal tissue destruction and a greater likelihood of tooth loss, highlighting the role of inadequate metabolic control in periodontal breakdown [6,7].

Increasing evidence suggests that both DM and periodontitis are associated with structural and functional alterations in the oral epithelium. Chronic hyperglycemia and persistent periodontal inflammation are known to induce sustained inflammatory and metabolic stress, which may disrupt epithelial homeostasis and lead to cellular injury [8,9]. Although periodontal pathology primarily involves gingival tissues, inflammatory and metabolic influences are not restricted to the gingiva and may extend to other oral mucosal sites [9,10].

Buccal epithelial cells are non-keratinized epithelial cells characterized by a high turnover rate, making them particularly sensitive to cellular stress and injury. Cytological assessment of these cells provides valuable information on nuclear and cellular characteristics, including nuclear size, nuclear-to-cytoplasmic ratio, and structural nuclear alterations [11,12]. Therefore, buccal mucosal cytology has been widely used as a non-invasive method to detect early cellular changes associated with systemic and chronic inflammatory conditions [13]. Among the various cellular alterations, the micronucleus assay is widely accepted as a robust indicator of genomic instability. Micronuclei arise from acentric chromosome fragments or entire chromosomes that fail to be incorporated into the daughter nuclei during mitosis, thereby reflecting the cumulative cytogenetic damage sustained by epithelial tissues over time [14]. Chronic hyperglycemia and periodontal inflammation have each been associated with oxidative stress, nuclear abnormalities, and increased micronucleus frequency [15–18]. Previous buccal cytology studies have generally examined diabetes or glycemic control [1,19,20] and periodontitis [10,21,22] as separate exposures. However, diabetes and periodontitis frequently coexist and are linked through bidirectional inflammatory and metabolic interactions [23]. Although these relationships are well established at the clinical and periodontal tissue levels, the cytological characteristics of clinically normal buccal mucosa across different combinations of glycemic control and periodontal disease severity remain insufficiently characterized. As the buccal mucosa is anatomically distinct from the gingival tissues directly affected by periodontitis, its evaluation may provide a complementary perspective on epithelial cellular alterations in individuals with coexisting metabolic and periodontal disease. Simultaneous stratification by HbA1c category and periodontitis stage therefore allows the characterization of patterns of nuclear abnormalities and cytomorphometric alterations across different clinical profiles.

Therefore, this cross-sectional study aimed to compare nuclear abnormalities and cytomorphometric characteristics of exfoliated buccal epithelial cells across clinical groups defined jointly by glycemic status and periodontitis stage. By integrating cytogenetic and cytomorphometric assessments, the study sought to characterize patterns of epithelial cel-

lular alterations in individuals with differing metabolic and periodontal profiles, including those with coexisting type 2 diabetes and advanced periodontitis.

2. Materials and Methods

2.1. Study Design and Ethical Approval

This observational, analytical cross-sectional study was approved by the Cankiri Karatekin University Health Sciences Ethics Committee (Decision No: 05.02.2024/11). All procedures were conducted in accordance with the ethical principles of the 1975 Declaration of Helsinki (revised in 2013). Prior to study inclusion, written informed consent was obtained from each participant. The study is reported in accordance with the STROBE guidelines.

2.2. Participants and Study Groups

Participants were consecutively recruited from patients presenting to the Department of Periodontology who met the eligibility criteria during the study period. The final sample size was determined by the number of eligible and consenting participants enrolled during this period. To be eligible for inclusion, individuals were required to be aged $\geq$ 35 years and met the criteria for either periodontitis or periodontal health according to the 2018 Classification of Periodontal and Peri-Implant Diseases and Conditions [4]. Individuals with type 2 DM were eligible if a medical diagnosis of type 2 DM was documented and confirmed by an internal medicine specialist (A.D.K.). Glycemic control was determined using contemporaneous HbA1c values obtained from medical records within the preceding month, reflecting metabolic status at the time of clinical examination. Participants without diabetes were required to have HbA1c < 5.7% and no uncontrolled or major systemic disorder; individuals with stable, well-controlled conditions not expected to substantially affect epithelial or periodontal homeostasis, such as well-controlled essential hypertension on stable monotherapy, were eligible. The exclusion criteria for all participants were: use of removable partial or complete dentures; active dental caries, sharp broken teeth, fractured restorations, or overhanging margins in proximity to the buccal sampling site; oral mucosal lesions, ulcerations, or frictional keratosis; self-reported severe xerostomia or clinical signs of salivary gland hypofunction; routine use of topical oral medications or antiseptic mouthwashes (e.g., chlorhexidine) within the preceding 4 weeks; type 1 DM; history of head and neck radiotherapy or malignancy; periodontal intervention within the preceding 6 months; regular use of medications known to alter cellular turnover or mucosal morphology (such as systemic antibiotics, corticosteroids, or immunosuppressive therapy within the past 3 months); pregnancy or lactation; current or former tobacco or alcohol consumption; diagnosed anemia, vitamin B12, or folate deficiency; and any uncontrolled chronic systemic conditions.

A total of 156 individuals were included in the study. Following eligibility assessment, participants were allocated into seven observed clinical groups according to their periodontal status and glycemic category. Participants with type 2 diabetes and HbA1c $\geq$ 7% were classified as having Stage I–II periodontitis (n = 28) or Stage III–IV periodontitis (n = 26), whereas participants with type 2 diabetes and HbA1c < 7% were classified as having Stage I–II periodontitis (n = 25) or Stage III–IV periodontitis (n = 25). Participants without diabetes comprised Stage I–II periodontitis (n = 16), Stage III–IV periodontitis (n = 16), and a periodontally healthy reference group (n = 20).

2.3. Buccal Cell Sampling and Cytological Analysis

Exfoliated buccal epithelial cells were collected prior to periodontal indexing to avoid procedure-related cellular alterations. To minimize potential confounding factors, partici-

pants were instructed to refrain from eating, drinking (except water), toothbrushing, and mouthrinse use for at least 2 h before sampling. All samples were collected between 09:00 and 12:00 h to reduce diurnal variation.

Buccal cell sampling was performed from the mid-region of the buccal mucosa using a pre-moistened wooden spatula with ten gentle scraping motions. The mid-buccal mucosa was selected as a standardized site to assess cellular responses in clinically normal tissue. Collected cells were immediately smeared onto clean microscope slides, air-dried, and fixed in 95% ethanol. The slides were subsequently stained with Giemsa stain for cytological evaluation.

To eliminate observer bias, all cytological slides were coded and anonymized prior to analysis. All microscopic examinations and morphometric evaluations were conducted by a single experienced pathologist (S.S.) with more than 10 years of experience, who was strictly blinded to the periodontal and glycemic status of the participants. For cytogenetic and cellular anomaly scoring, 1000 intact epithelial cells per participant were evaluated under a light microscope (Olympus CX41, Olympus Corporation, Tokyo, Japan) using ×40 and ×100 objectives. Micronuclei were identified according to established morphological criteria [14]. The numbers of micronuclei, binucleated cells, and cells with perinuclear halo were recorded and expressed per 1000 cells. Inflammation was recorded as present when inflammatory cells accompanied the epithelial cells on the smear and as absent otherwise.

For quantitative cytomorphometric analysis, high-resolution digital photomicrographs were captured using an Olympus SC100 digital camera (3840 × 2748 pixels; Olympus Soft Imaging Solutions, Münster, Germany) mounted on the microscope with a 10× objective (numerical aperture 0.25). Morphometric measurements were performed using calibrated image analysis software (Olympus cellSens, Olympus Soft Imaging Solutions, Münster, Germany). Nuclear area (NA, μm^2) and cytoplasmic area (CA, μm^2) were outlined in 50 clearly defined, non-overlapping epithelial cells per participant, and the nuclear-to-cytoplasmic (N/C) ratio was calculated as NA/CA. To evaluate intra-examiner reproducibility, micronucleus scoring and morphometric measurements were repeated in 10 randomly selected cases 15 days after the initial evaluation, demonstrating good reliability (intraclass correlation coefficient, ICC = 0.87 for micronucleus counts and 0.90 for NA, CA, and N/C ratio). Representative photomicrographs illustrating micronucleus formation, inflammatory cell infiltration, and multiple nuclear abnormalities in exfoliated buccal epithelial cells are shown in Figure 1.

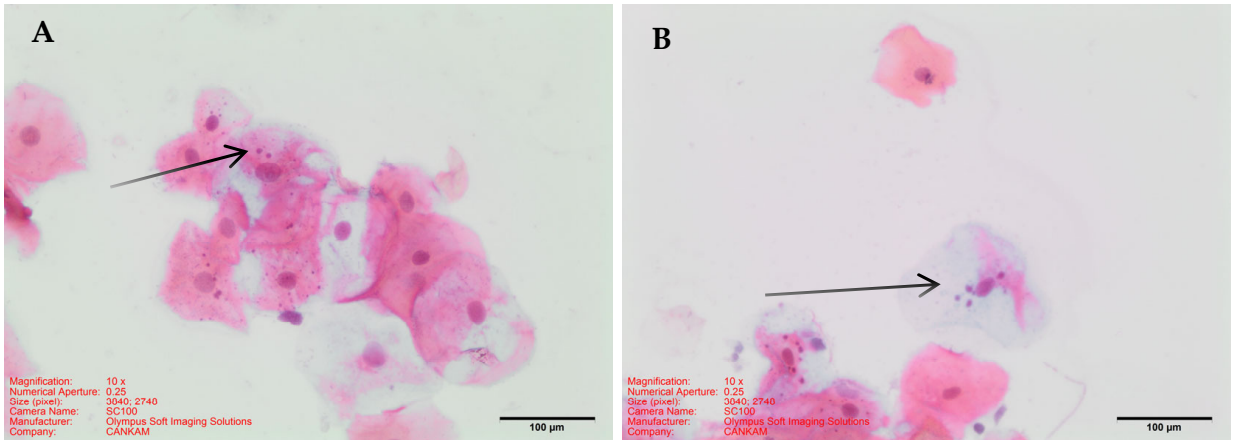


Figure 1. *Cont.*

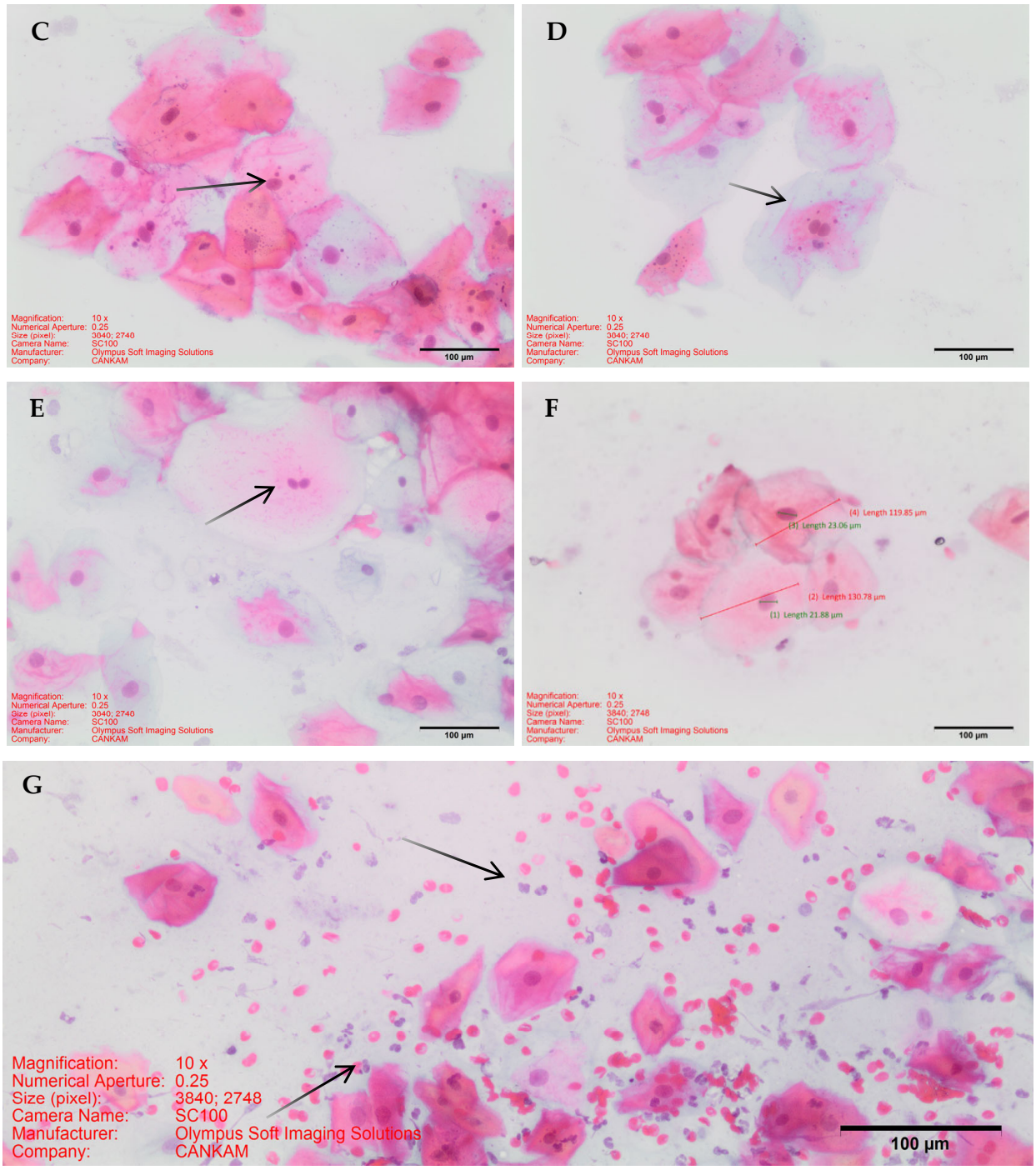


Figure 1. Representative cytological and cytomorphometric findings in exfoliated buccal epithelial cells. (**A**–**C**) Micronuclei (arrows) adjacent to the main nuclei of epithelial cells (Giemsa staining, original magnification ×400). (**D**,**E**) Nuclear abnormalities, including binucleated cells (arrows) (Giemsa staining, original magnification ×400). (**F**) Measurement of nuclear (green) and cytoplasmic (red) dimensions for cytomorphometric assessment (original magnification ×400). (**G**) Inflammatory cells (arrows) accompanying exfoliated epithelial cells (Giemsa staining, original magnification ×200).

2.4. Periodontal Examination and Calibration

All participants underwent a comprehensive full-mouth periodontal examination performed by a single periodontist (B.K.) using a standard periodontal probe (UNC-15, Hu-Friedy, Chicago, IL, USA). Probing pocket depth (PPD) and gingival margin position were recorded at six sites per tooth (mesiobuccal, mid-buccal, distobuccal, mesiolingual,

mid-lingual, and distolingual) for all present teeth, excluding third molars; clinical attachment level (CAL) was calculated accordingly. Bleeding on probing (BOP) was evaluated dichotomously (presence/absence) within 15 s following probing [24]. To evaluate intra-examiner measurement reliability, duplicate measurements were performed 15 days apart in the first 10 consecutive participants. The intra-examiner ICC demonstrated good reproducibility, with values of 0.89 for PPD and 0.87 for CAL. Periapical and panoramic radiographs obtained as part of routine clinical care prior to the study examination were used to assess alveolar bone loss; no radiographs were taken for research purposes. For all participants, data collection was conducted within a single baseline visit in a standardized sequence: buccal epithelial cell sampling was performed first to prevent procedure-related bleeding or mechanical trauma, followed by comprehensive clinical periodontal examination, and subsequent recording of contemporaneous glycemic data (with the second visit in the calibration subset restricted solely to duplicate periodontal charting).

2.5. Periodontal Diagnosis and Case Staging

The periodontal status was categorized in accordance with the 2018 Classification of Periodontal and Peri-Implant Diseases and Conditions [4]. The reference group comprised participants without diabetes (HbA1c < 5.7%) who had an intact periodontium, PPD $\leq$ 3 mm, BOP < 10%, and no clinical attachment loss or radiographic bone loss. Periodontitis was diagnosed by interdental CAL at $\geq$2 non-adjacent teeth, and participants were categorized as Stage I–II or Stage III–IV based on clinical and radiographic parameters. Stage allocation was performed independently by two experienced periodontists (B.K., A.E.); initial disagreements were resolved by consensus.

2.6. Statistical Analysis

Statistical analyses were performed using R version 4.4.3 (R Foundation for Statistical Computing, Vienna, Austria) within the RStudio environment (version 2026.1.0.392; Posit Software, Boston, MA, USA). Participants were classified into seven groups according to glycemic category (without diabetes, HbA1c < 7%, or HbA1c $\geq$ 7%) and periodontal status (healthy, Stage I–II, or Stage III–IV). Age was categorised as <50 and $\geq$50 years, and diabetes duration as 1–5, 6–10 and >10 years. The age cutoff of 50 years was selected considering the age distribution and clinical characteristics of the study population, which consisted of adults aged 35 years and older and included individuals with type 2 diabetes. Because periodontally healthy participants with diabetes were not included, glycemic category and periodontal status were combined into a single seven-level group variable. Micronuclei, binucleated cells and perinuclear halo were analysed as count data, inflammation as a binary variable, and nuclear area, cytoplasmic area and N/C ratio as continuous variables. Descriptive statistics were expressed as mean $\pm$ standard deviation or number (percentage).

Each parameter was compared across the seven groups using a regression model adjusted for age group and sex. Age and sex were selected as covariates because of their potential associations with buccal epithelial characteristics, while the eligibility criteria were designed to minimize confounding from relevant systemic conditions, medication use, and local oral factors. Count variables were analysed with Poisson regression, replaced by negative binomial regression when a likelihood ratio test indicated overdispersion; when a group contained no events, bias-reduced Poisson regression with dispersion-scaled standard errors was applied. Inflammation was analysed with bias-reduced logistic regression. Continuous variables were log-transformed and analysed with linear regression using heteroscedasticity-consistent (HC3) standard errors. Overall group differences were assessed with joint Wald tests. Pairwise comparisons of estimated marginal means were

performed with Šidák correction, and results are reported as rate ratios, odds ratios or ratios of geometric means with Šidák-adjusted 95% confidence intervals (CI).

The effect of diabetes duration was evaluated in patients with diabetes using the same models, additionally adjusted for HbA1c level and periodontal status. Model fit was checked using simulation-based residual diagnostics for count and binary outcomes and residual plots for continuous outcomes. A sensitivity power analysis indicated that the sample size allowed detection of effect sizes of f = 0.36 for comparisons among the seven groups and f = 0.34 for comparisons among diabetes duration groups ($\alpha = 0.05$, power = 0.80).

Analyses were performed with the R packages MASS (7.3-65), brglm2 (1.0.1), emmeans (2.0.1), sandwich (3.1-1), multcomp (1.4-29), DHARMa (0.4.7) and pwr (1.3-0). A two-sided p value < 0.05 was considered statistically significant.

3. Results

Of the 156 participants included in the study, 52.6% ($n = 82$) were female and 47.4% ($n = 74$) were male. The distribution of antidiabetic treatment and systemic comorbidities among participants with type 2 diabetes is presented in Table 1. Metformin was the most frequently used antidiabetic medication, while hypertension was the most commonly reported systemic comorbidity. All between-group comparisons were adjusted for age group and sex.

Table 1. Distribution of antidiabetic treatment and systemic comorbidities among participants with type 2 diabetes according to glycemic control and periodontal stage.

Characteristics, *n* (%)	HbA1c < 7% Stage I–II (*n* = 25)	HbA1c < 7% Stage III–IV (*n* = 25)	HbA1c ≥ 7% Stage I–II (*n* = 28)	HbA1c ≥ 7% Stage III–IV (*n* = 26)
Antidiabetic treatment				
Metformin only	22 (88.0)	21 (84.0)	22 (78.6)	20 (76.9)
Insulin only	2 (8.0)	1 (4.0)	5 (17.9)	2 (7.7)
Metformin + insulin	0 (0.0)	1 (4.0)	1 (3.6)	3 (11.5)
Diet only	1 (4.0)	2 (8.0)	0 (0.0)	1 (3.8)
Systemic comorbidities				
Hypertension	8 (32.0)	11 (44.0)	10 (35.7)	14 (53.8)
Cardiac disease, unspecified	0 (0.0)	1 (4.0)	0 (0.0)	0 (0.0)
Rheumatic disease, unspecified	0 (0.0)	0 (0.0)	0 (0.0)	1 (3.8)
No recorded comorbidity	17 (68.0)	13 (52.0)	18 (64.3)	11 (42.3)

Values are presented as *n* (%). Percentages were calculated using the total number of participants in each group. HbA1c, glycated hemoglobin.

3.1. Comparison of Cytological Parameters According to HbA1c Level and Periodontal Status

Descriptive statistics and the results of pairwise comparisons are presented in Table 1, and model-based estimates are shown in Figure 2. Significant overall differences were observed for all evaluated parameters (Table 2).

Micronuclei counts were highest in the HbA1c ≥ 7% groups (Table 2, Figure 2). Compared with the reference group, counts were higher in the HbA1c ≥ 7%/Stage I–II group (ratio 12.96, 95% CI: 5.95–28.25) and the HbA1c ≥ 7%/Stage III–IV group (ratio 11.52, 95% CI: 5.30–25.04; both $p < 0.001$). Both HbA1c ≥ 7% groups also had higher counts than all groups without diabetes and the HbA1c < 7%/Stage III–IV group (Table 2). Counts were also higher in the HbA1c < 7%/Stage I–II (ratio 7.07, 95% CI: 3.20–15.58, $p < 0.001$) and HbA1c < 7%/Stage III–IV groups (ratio 3.95, 95% CI: 1.74–8.93, $p < 0.001$) than in the reference group. Among participants without diabetes, counts were higher in the Stage III–IV group than in the reference group (ratio 2.54, 95% CI: 1.04–6.17, $p = 0.031$). The HbA1c < 7%/Stage I–II group did not differ significantly from the HbA1c ≥ 7% groups.

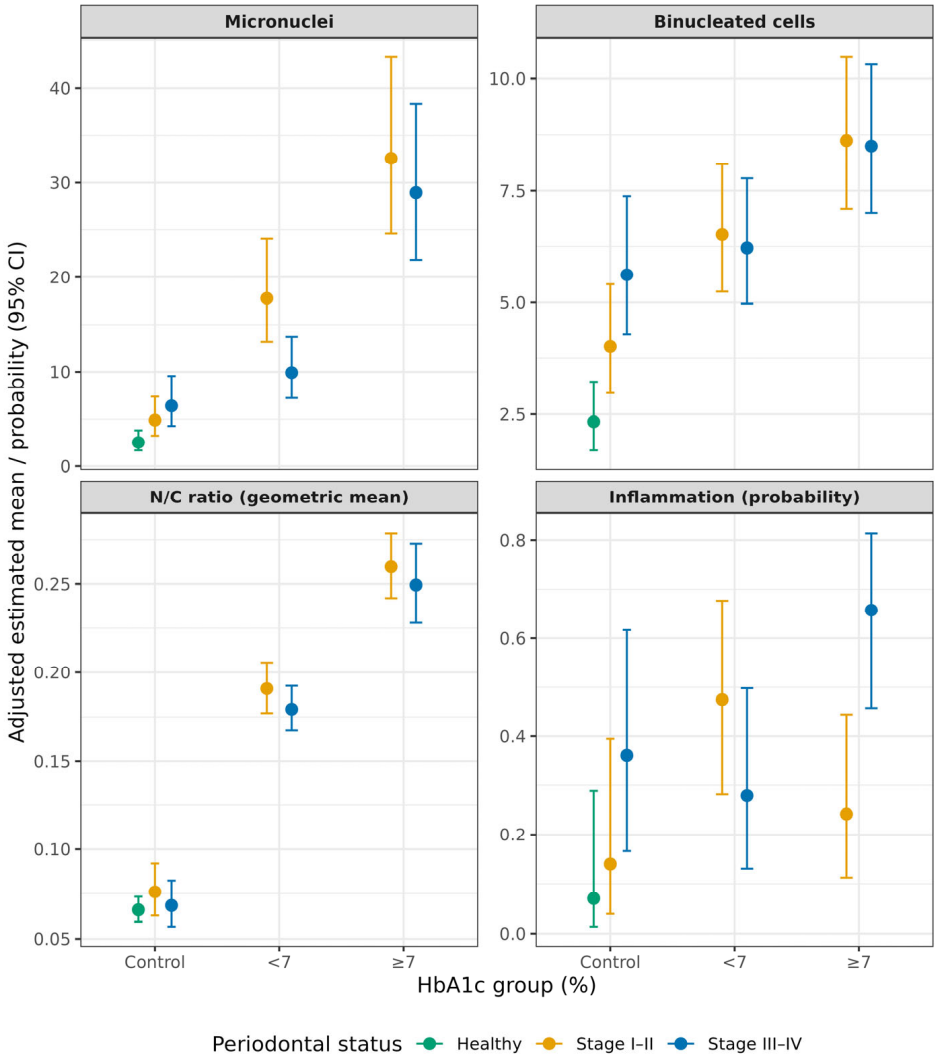


Figure 2. Adjusted estimated means (95% CI) of micronuclei, binucleated cells and nuclear-to-cytoplasmic (N/C) ratio, and adjusted probability of inflammation, according to glycemic category (no diabetes, HbA1c < 7%, HbA1c ≥ 7%) and periodontal status (healthy, Stage I–II, Stage III–IV). Estimates were derived from models adjusted for age group and sex: negative binomial regression for micronuclei and binucleated cells, log-linear regression for N/C ratio (geometric means) and bias-reduced logistic regression for inflammation. Periodontally healthy participants were included only among participants without diabetes.

Binucleated cell counts were lowest in the reference group and highest in the HbA1c ≥ 7% groups (Table 2, Figure 2). Compared with the reference group, counts were higher in the HbA1c ≥ 7%/Stage I–II group (ratio 3.70, 95% CI: 2.05–6.67) and the HbA1c ≥ 7%/Stage III–IV group (ratio 3.65, 95% CI: 2.03–6.55; both $p < 0.001$). Both HbA1c ≥ 7% groups also had higher counts than the Stage I–II group without diabetes (ratios 2.15, 95% CI: 1.23–3.76, and 2.12, 95% CI: 1.22–3.69, respectively; both $p < 0.001$). Counts in both HbA1c < 7% groups and in the Stage III–IV group without diabetes (ratio 2.41, 95% CI: 1.26–4.61, $p < 0.001$) were also higher than in the reference group.

The overall test indicated a difference in perinuclear halo across groups ($p = 0.015$); however, this difference could not be attributed to any specific pair of groups, as no pairwise comparison remained significant after correction for multiple comparisons (Table 2).

Table 2. Oral cytological parameters and inflammation according to HbA1c level and periodontal status.

Glycemic Category	Periodontal Status	*n*	Binucleated Cells	Micronuclei	Perinuclear Halo	Inflammation, *n* (%)	Nuclear Area	Cytoplasmic Area	N/C Ratio
No diabetes	Periodontally Healthy	20	2.40 ± 1.50 ^{a}	2.55 ± 1.47 ^{a}	0.00 ± 0.00	1 (5.0) ^{a}	9.32 ± 0.74 ^{a}	143.29 ± 28.73 ^{b}	0.068 ± 0.016 ^{a}
No diabetes	Stage I–II	16	4.19 ± 3.25 ^{ab}	5.12 ± 5.10 ^{ab}	0.25 ± 0.58	2 (12.5) ^{ab}	11.45 ± 1.99 ^{b}	153.37 ± 39.32 ^{b}	0.081 ± 0.028 ^{a}
No diabetes	Stage III–IV	16	5.94 ± 1.69 ^{bc}	6.44 ± 7.45 ^{b}	1.31 ± 0.87	6 (37.5) ^{ab}	10.87 ± 1.64 ^{ab}	160.25 ± 39.58 ^{b}	0.074 ± 0.031 ^{a}
<7%	Stage I–II	25	6.52 ± 2.71 ^{bc}	20.12 ± 21.01 ^{cd}	0.24 ± 0.60	12 (48.0) ^{ab}	19.91 ± 2.63 ^{c}	106.03 ± 12.06 ^{a}	0.190 ± 0.036 ^{b}
<7%	Stage III–IV	25	5.84 ± 2.82 ^{bc}	9.24 ± 5.64 ^{bc}	0.52 ± 0.87	6 (24.0) ^{ab}	19.39 ± 2.84 ^{c}	107.33 ± 6.72 ^{a}	0.182 ± 0.032 ^{b}
≥7%	Stage I–II	28	8.18 ± 6.16 ^{c}	33.79 ± 29.21 ^{d}	0.57 ± 1.60	6 (21.4) ^{ab}	29.68 ± 2.93 ^{d}	116.48 ± 16.01 ^{a}	0.259 ± 0.043 ^{c}
≥7%	Stage III–IV	26	8.31 ± 3.95 ^{c}	29.15 ± 21.74 ^{d}	0.69 ± 1.05	17 (65.4) ^{b}	28.52 ± 5.86 ^{d}	114.96 ± 25.61 ^{a}	0.253 ± 0.051 ^{c}
p			<0.001	<0.001	0.015	0.002	<0.001	<0.001	<0.001

Values are mean ± SD or *n* (%). Within each column, groups sharing a superscript letter do not differ significantly (pairwise comparisons of estimated marginal means from models adjusted for age group and sex, Šidák correction); letters are omitted when no pairwise difference remained significant after correction. p: overall group difference (joint Wald test, adjusted for age group and sex). Count parameters: negative binomial or bias-reduced Poisson regression; inflammation: bias-reduced logistic regression; nuclear area, cytoplasmic area and N/C ratio: log-linear regression with HC3 robust standard errors. N/C: nuclear-to-cytoplasmic ratio. No diabetes: participants without diabetes (HbA1c < 5.7%). Reference group: periodontally healthy participants without diabetes.

The proportion of participants with inflammation ranged from 5.0% in the reference group to 65.4% in the HbA1c ≥ 7%/Stage III–IV group (Table 2, Figure 2). The only significant pairwise difference was observed between these two groups (OR = 25.60, 95% CI: 1.39–473.19, $p = 0.016$).

Nuclear area was significantly larger in all groups with diabetes than in all groups without diabetes ($p < 0.001$) and larger in the HbA1c ≥ 7% groups than in the HbA1c < 7% groups (ratios 1.39–1.57, $p < 0.001$). Among participants without diabetes, nuclear area was larger in the Stage I–II group than in the reference group (ratio 1.22, 95% CI: 1.05–1.41, $p = 0.001$). Cytoplasmic area was smaller in all groups with diabetes than in all groups without diabetes (ratios 0.68–0.82, $p \leq 0.029$), with no differences among the groups with diabetes or among the groups without diabetes (Table 2). The N/C ratio was progressively higher from the groups without diabetes to the HbA1c < 7% groups (ratios 2.36–2.89, $p < 0.001$) and from the HbA1c < 7% groups to the HbA1c ≥ 7% groups (ratios 1.31–1.45, $p < 0.001$), whereas it did not differ according to periodontal status within any glycemic category (Table 2, Figure 2).

3.2. Effect of Diabetes Duration on Cytological Parameters and Inflammation

Among patients with diabetes ($n = 104$), diabetes duration was not significantly associated with binucleated cells ($p = 0.076$), micronuclei ($p = 0.840$), perinuclear halo ($p = 0.117$), inflammation ($p = 0.813$), nuclear area ($p = 0.211$) or cytoplasmic area ($p = 0.499$) after adjustment for HbA1c level, periodontal status, age group and sex (Table 3). An isolated borderline overall difference was observed for the N/C ratio ($p = 0.047$), with the only significant pairwise difference being a higher N/C ratio in the 6–10-year group than in the >10-year group (ratio 1.11, 95% CI: 1.000–1.223, $p = 0.049$) (Table 3). Given the number of evaluated outcomes and the borderline estimates, this finding was interpreted cautiously.

Table 3. Oral cytological parameters and inflammation according to diabetes duration in patients with diabetes (n = 104).

Diabetes Duration (Years)	*n*	Binucleated Cells	Micronuclei	Perinuclear Halo	Inflammation, *n* (%)	Nuclear Area	Cytoplasmic Area	N/C Ratio
1–5	29	5.79 ± 3.14	23.34 ± 22.96	0.62 ± 0.86	12 (41.4)	25.00 ± 5.50	115.34 ± 23.04	0.220 ± 0.047 [ab]
6–10	33	7.76 ± 3.71	24.45 ± 28.31	0.24 ± 0.61	14 (42.4)	24.76 ± 5.78	107.84 ± 15.66	0.232 ± 0.051 [b]
>10	42	7.86 ± 5.17	22.71 ± 19.07	0.64 ± 1.48	15 (35.7)	24.11 ± 6.75	111.45 ± 12.58	0.217 ± 0.060 [a]
p		0.076	0.840	0.117	0.813	0.211	0.499	0.047

Values are mean ± SD or *n* (%). *p*: overall effect of diabetes duration adjusted for HbA1c level, periodontal status, age group and sex (joint Wald test). Superscript letters are shown only when $p < 0.05$ and at least one pairwise difference remained significant after Šidák correction; groups sharing a letter do not differ significantly. N/C: nuclear-to-cytoplasmic ratio.

4. Discussion

The present cross-sectional study compared cytomorphometric characteristics and nuclear abnormalities in exfoliated buccal epithelial cells across seven clinical groups defined by glycemic category and periodontal stage. Adjusted group differences were observed for micronuclei, binucleated cells, nuclear area, cytoplasmic area, and the N/C ratio. Within the observed clinical strata, differences were generally more consistent across glycemic categories than between grouped periodontal stages within the same glycemic category. However, because periodontally healthy participants were included only among individuals without diabetes, these patterns should not be interpreted as evidence of a greater independent contribution of glycemic status relative to periodontal severity. Rather, the findings characterize cytological variation across the specific combinations of glycemic and periodontal status represented in the study population.

Micronuclei in exfoliated buccal epithelial cells have been widely evaluated as cytological indicators of chromosomal alterations [14,21]. In agreement with prior literature [1,19,20], the highest micronucleus counts in the present sample were generally observed in the HbA1c $\geq$ 7% groups. Oxidative stress, impaired DNA repair, and chromosomal missegregation have been proposed as possible biological explanations for increased micronucleus frequency in diabetes [15,25,26]. Nevertheless, the present cross-sectional findings do not demonstrate these mechanisms directly. Moreover, the absence of a significant difference between the HbA1c < 7%/Stage I–II group and the HbA1c $\geq$ 7% groups indicates that the observed pattern was not uniformly graded across all clinical strata.

Micronuclei and binucleated cells represent related but distinct nuclear endpoints. Micronuclei are cytogenetic markers associated with structural or numerical chromosomal abnormalities, whereas binucleated cells may reflect altered cytokinesis or cytotoxic stress. Both outcomes were most frequent in the HbA1c $\geq$ 7% groups. Among participants without diabetes, the Stage III–IV group also had higher micronucleus and binucleated cell counts than the periodontally healthy reference group. This finding is consistent with previous reports of increased nuclear abnormalities in periodontal disease [12,17,21]. These findings should therefore be interpreted as associations within the examined clinical groups rather than as evidence of independent effects of glycemic or periodontal status.

Perinuclear halo formation showed an overall difference across groups, but no individual pairwise comparison remained significant after correction for multiple testing. Inflammatory cells were most frequent in the HbA1c $\geq$ 7%/Stage III–IV group, and the only significant pairwise difference was between this group and the reference group. The wide confidence interval for this comparison indicates substantial imprecision. Given that only one pairwise comparison was significant and its confidence interval was wide, the inflammatory-cell finding should be interpreted cautiously.

Regarding quantitative morphometry, the N/C ratio was higher in groups with diabetes than in groups without diabetes and was highest in the HbA1c $\geq$ 7% groups. This pattern reflected larger nuclear areas and smaller cytoplasmic areas across the observed clinical strata. Previous studies have similarly reported larger nuclear dimensions and higher N/C ratios in patients with diabetes [27,28]. Although associations between periodontal clinical parameters and cellular dimensions have been reported in the literature [29], no significant differences in the N/C ratio were observed between periodontal stages within the same glycemic category in the present study. Accordingly, glycemic status should be taken into account when evaluating buccal cytomorphometric parameters in periodontal research. However, the absence of periodontally healthy individuals with diabetes in our study limits the ability to assess the independent associations of glycemic status and periodontal disease severity with cytomorphometric alterations. Therefore, the observed differences should not be interpreted as indicating that glycemic status has a greater influence on cytomorphometric alterations than periodontal disease severity. In particular, studies comparing patients with periodontitis and periodontally healthy controls should consider potential differences in glycemic status to avoid attributing cytomorphometric variations solely to periodontal disease.

Several cytomorphometric investigations have demonstrated significant enlargement of the nuclear area in diabetic buccal mucosa relative to controls without diabetes [30,31]. Other studies have described variations in cytoplasmic dimensions and inverse cytoplasmic-to-nuclear relationships across different metabolic contexts [30,32]. The association of HbA1c with cellular ratios reported in earlier studies [32] is compatible with the group pattern observed here. However, differences in sampling, glycemic distributions, staining techniques, and morphometric methods limit direct comparisons. Studies by Seifi et al. [13] and Mirescu et al. [33] also found no significant cytomorphometric differences between type 1 and type 2 diabetes, further illustrating the heterogeneity of findings across diabetic populations.

After adjustment for HbA1c category, periodontal status, age group, and sex, diabetes duration showed no consistent association with the evaluated cytological outcomes. The isolated borderline difference in the N/C ratio between the 6–10-year and >10-year groups should be interpreted cautiously because it was not accompanied by consistent differences in the other outcomes. Previous studies have likewise reported inconsistent findings regarding diabetes duration and buccal cytological alterations [1,3,19,34]. Differences in study populations, treatment patterns, glycemic exposure, and the categorization of diabetes duration may contribute to this heterogeneity [26]. In the present study, differences were more apparent across HbA1c categories than across diabetes-duration categories; however, this pattern should be interpreted within the context of the study sample rather than as evidence that current glycemic control has a stronger biological effect than diabetes duration.

To our knowledge, few studies have evaluated buccal nuclear abnormalities and digital cytomorphometry concurrently across strata defined by both HbA1c category and periodontitis within a single cohort. A key strength of the present study is this integrated, stratified evaluation of DM and periodontitis using contemporary diagnostic criteria [4], combined with blinded cytological assessment and quantitative digital morphometry. The inclusion of a periodontally healthy reference group without diabetes also provided a clinically interpretable basis for the observed group comparisons. Buccal cytology may offer a complementary, minimally invasive approach to assessing epithelial alterations that are not directly apparent during routine periodontal examination. However, in light of established clinical measures such as HbA1c and conventional periodontal parameters, the translational relevance and clinical utility of exfoliative cytology remain speculative.

Rather than serving as an immediate surrogate for established diagnostic tools, cytogenetic and cytomorphometric alterations reflect cellular-level genotoxicity, accelerated senescence, and generalized epithelial vulnerability under concurrent systemic and local inflammatory stress. Before these cytological parameters can be considered clinically viable biomarkers, rigorous validation is required; specifically, prospective longitudinal studies demonstrating responsiveness to periodontal and glycemic interventions, the establishment of validated discriminatory diagnostic thresholds, and evidence correlating cellular damage with actual disease progression.

This study has limitations. Its cross-sectional design precludes conclusions regarding temporal or causal relationships. Although the reference group facilitated the interpretation of the observed group differences, the absence of periodontally healthy participants with diabetes limited a full factorial evaluation of glycemic and periodontal status. Consequently, the independent contributions of glycemic status and periodontal severity, as well as their potential interaction, could not be fully evaluated. Group sizes were also modest and unequal, and the sensitivity power analysis indicated that only moderate-to-large differences could be detected. Therefore, non-significant pairwise comparisons, particularly those involving smaller groups or imprecise estimates, should not be interpreted as evidence of no association. Categorizing age may have resulted in some loss of information and residual age-related confounding. In addition, residual confounding by clinical or metabolic factors cannot be entirely excluded, as BMI was not recorded and antidiabetic treatment and systemic comorbidities were not included in the adjusted models. Furthermore, cytological sampling was restricted to a single buccal mucosal site, limiting the generalizability of the findings to other oral mucosal regions.

5. Conclusions

Nuclear abnormalities and cytomorphometric characteristics of exfoliated buccal epithelial cells varied across clinical groups defined by glycemic status and periodontal stage. Within the clinical groups examined, these differences appeared more consistent across glycemic categories, whereas variation between grouped periodontal stages was less pronounced. These findings suggest that glycemic status may be relevant to buccal epithelial cytomorphology in individuals with different periodontal stages. Further longitudinal studies may help clarify the temporal relationships and clinical relevance of these cytological alterations.

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Data Availability Statement: The datasets generated and/or analyzed during the current study are not publicly available due to ethical and privacy considerations but are available from the corresponding author on reasonable request.

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Abbreviations

The following abbreviations are used in this manuscript:

DM	Diabetes Mellitus
HbA1c	Hemoglobin A1c
NA	Nuclear area
CA	Cytoplasmic area
N/C	Nuclear-to-cytoplasmic ratio
PPD	Probing pocket depth
CAL	Clinical attachment level
BOP	Bleeding on Probing

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