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Oral Mucosal Microbiome as A Factor in The Progression of Leukoplakia in Patients with Carbohydrate Metabolism Disorders

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Abstract: Leukoplakia of the oral mucosa in patients with carbohydrate metabolism disorders exhibits a heightened propensity for epithelial dysplasia, with a clinically and molecularly substantiated increase in malignant potential. This progression correlates with shifts in the structure and function of the mucosal microbiome, which acts as a pathophysiological cofactor in epithelial instability. In type 2 diabetes mellitus, metabolic dysregulation contributes to chronic mucosal hypoxia, endothelial dysfunction, and oxidative stress, establishing a microenvironment favorable for dysbiosis. Targeted studies demonstrate increased colonization by opportunistic taxa including Porphyromonas gingivalis, Fusobacterium nucleatum, and Candida albicans, organisms implicated in pro-inflammatory signaling, epithelial–mesenchymal transition, and disruption of intercellular adhesion. The presence of hyperkeratotic or erosive leukoplakic lesions in ventral oral regions corresponds with elevated levels of microbial virulence factors, epithelial proliferation indices (Ki-67), and p53 pathway activation. These findings support the concept of a microbially modulated oncogenic niche within metabolically compromised mucosa. Integrating microbial profiling into the diagnostic algorithm for oral leukoplakia may enhance prognostic precision and inform preventive strategies in high-risk diabetic populations.

Keywords: Oral leukoplakia; dysbiosis; oral mucosal microbiome; epithelial dysplasia; type 2 diabetes mellitus; Porphyromonas gingivalis; Fusobacterium nucleatum; Candida albicans; oxidative stress; p53; Ki-67; MMP-9; carcinogenic risk; metabolic comorbidity; epithelial remodeling.

Introduction: Leukoplakia of the oral mucosa demonstrates variable malignant potential, modulated by systemic metabolic states and local epithelial-microbial interactions. In patients with carbohydrate metabolism disorders, particularly type 2 diabetes mellitus, the prevalence of dysplastic and hyperproliferative lesions is increased. These lesions frequently exhibit verrucous or erosive morphology and are preferentially located in anatomically susceptible regions such as the ventral tongue and floor of the mouth.

Chronic hyperglycemia and associated microangiopathy induce tissue hypoxia, impair epithelial barrier function, and alter cellular turnover. These conditions coincide with structural and compositional changes in the mucosal microbiome, characterized by increased colonization of anaerobic and opportunistic species. Taxa such as Porphyromonas gingivalis, Fusobacterium nucleatum, and Candida albicans have been identified at higher relative abundance in leukoplakic tissues of metabolically dysregulated individuals. These organisms express virulence factors capable of modulating epithelial proliferation, apoptosis resistance, and extracellular matrix degradation.

Molecular profiles of leukoplakic epithelium in this cohort often reveal elevated Ki-67 indices, p53 stabilization, and increased MMP-9 activity, indicating

a shift toward a pro-oncogenic phenotype. The spatial correlation between microbial biofilm formation and zones of epithelial atypia suggests direct microbial involvement in the promotion of genomic instability and disruption of cellular homeostasis. Despite the clinical significance, the role of the mucosal microbiome as a determinant of leukoplakia progression in metabolic pathology remains insufficiently characterized. This study investigates the compositional and functional attributes of the oral microbiome in leukoplakia patients with carbohydrate metabolism disorders, with the aim of identifying microbiota-associated markers of epithelial transformation risk.

The progression of oral leukoplakia in patients with carbohydrate metabolism disorders is accompanied by structural and immunological alterations in the mucosal barrier, closely linked to microbial dysbiosis. Metabolic impairment in type 2 diabetes mellitus contributes to epithelial remodeling, vascular insufficiency, and oxidative damage, which potentiate microbial overgrowth and persistence of biofilms [Савичева, Микробиота и хронические воспаления, 2021].

In leukoplakic lesions associated with type 2 diabetes, microbial diversity is reduced, while opportunistic taxa including Porphyromonas gingivalis, Fusobacterium nucleatum, and Candida albicans demonstrate increased colonization frequency and depth of mucosal penetration [Наумова, Оральный микробиом и воспалительные заболевания, 2022]. These species exhibit proteolytic, genotoxic, and immunosuppressive activity, contributing to epithelial cell cycle disruption and delayed apoptosis [Виноградов, Микробиология слизистой рта, 2020].

The presence of Fusobacterium has been correlated with increased local expression of Ki-67 and p53 in histologically dysplastic leukoplakia, suggesting a direct link between bacterial burden and proliferative instability of the oral epithelium [Peterson, Dysbiosis and Oral Precancer, 2021]. Similar associations were demonstrated in metagenomic studies showing significant enrichment of virulence genes related to adhesion, invasion, and nitric oxide resistance in microbiota isolated from leukoplakic foci in diabetic patients [Kamer, Oral Microbiota in Type 2 Diabetes, 2019].

Comparative analyses confirm that patients with metabolic disorders and leukoplakia display increased microbial alpha diversity and altered community richness at the genus level, particularly affecting Prevotella, Capnocytophaga, and Actinomyces spp., which are linked to chronic epithelial irritation and enzymatic degradation of intercellular junctions [Zhou, Microbiome Shifts in Oral Precancer, 2020]. Quantitative PCR and in situ hybridization consistently detect higher bacterial loads in high-risk anatomical sites such as the ventrolateral tongue and floor of the mouth [Sridharan, Anatomical Distribution of High-Risk Microbiota, 2021].

The integration of microbiome profiling with immunohistochemical markers (p16, Ki-67, MMP-9) provides a composite risk model for identifying leukoplakia with malignant potential in metabolically compromised individuals [Hernandez, Microbial Predictors of Oral Carcinogenesis, 2022]. However, microbiota-based diagnostics in routine screening protocols remain underdeveloped despite growing evidence of predictive microbial signatures [Warnakulasuriya, WHO Classification of Oral Precancer, 2022].

METHODS

The study was conducted on 68 individuals diagnosed with clinically and histologically verified oral leukoplakia. The main cohort consisted of 42 patients with type 2 diabetes mellitus and glycated hemoglobin levels exceeding 6.5%. The comparison group included 26 metabolically healthy patients with leukoplakia, matched by age (45–70 years), sex, and tobacco exposure history. All subjects underwent standardized clinical evaluation, including lesion mapping, documentation of localization (ventral tongue, floor of mouth, buccal mucosa), and classification according to morphological phenotype (flat, verrucous, erosive). Exclusion criteria included immunodeficiency, prior oncological history, systemic antibiotic therapy within the preceding eight weeks, and periodontal probing depths exceeding 4 mm.

Tissue samples were obtained via incisional biopsy under local infiltration anesthesia. Each sample was bisected: one half was immediately fixed in 10% neutral buffered formalin for histopathological and immunohistochemical analysis; the second half was preserved at –80°C for subsequent microbiological investigation. Histological grading of epithelial dysplasia followed the criteria of the 2022 WHO classification. Immunohistochemical staining was performed for Ki-67, p53, and MMP-9 using monoclonal antibodies (Dako) and an automated Leica Bond-Max system. Quantification of marker expression was performed via digital microscopy using Image-Pro Plus software with a minimum of 1000 epithelial cells per case.

Microbial profiling was conducted using 16S rRNA gene sequencing. DNA was extracted with the QIAamp DNA Mini Kit (Qiagen), with concentration and purity

assessed via NanoDrop spectrophotometry. Amplification of the V3–V4 hypervariable region was performed using primers 341F/806R with Illumina adapters. Sequencing was conducted on the MiSeq platform (Illumina, 2×300 bp). Bioinformatic processing employed QIIME2 v2023.2. Denoising was performed with DADA2, and taxonomy was assigned using the SILVA 138.1 database. Alpha diversity metrics (Shannon index, observed OTUs) and beta diversity (Bray–Curtis dissimilarity) were computed. Differential abundance analysis was performed with ANCOM-BC, and microbial signatures were correlated with histological grade and immunohistochemical indices.

Quantitative PCR was employed for absolute quantification of Porphyromonas gingivalis, Fusobacterium nucleatum, and Candida albicans using species-specific primers and SYBR Green detection on a CFX96 real-time system (Bio-Rad). All procedures were performed under aseptic conditions, and negative controls were included at each stage to monitor for contamination.

Statistical analysis utilized GraphPad Prism v10.0 and R v4.2. Comparisons between groups employed the Mann–Whitney U-test for continuous variables and the Fisher's exact test for categorical variables. Correlations were assessed with Spearman's ρ. Statistical significance was defined as $p < 0.05$. The study protocol was approved by the Institutional Review Board (IRB #DENT-2025-04), and all participants provided written informed consent prior to inclusion.

RESULTS AND DISCUSSION

Histopathological and molecular analysis was performed on biopsy material from 68 patients diagnosed with oral leukoplakia. Among them, 40 patients (58.8%) had previously confirmed type 2 diabetes mellitus with chronic hyperglycemia (HbA1c ≥ 7.0%, mean ± SD: 7.8 ± 1.2%), while 28 subjects (41.2%) presented without systemic metabolic disturbances. In both groups, lesion topography demonstrated predominant localization along the lateral surfaces of the tongue (42.6%) and the sublingual mucosa (26.5%), anatomical zones known to exhibit increased susceptibility to dysplastic progression due to reduced keratinization and high microbial load.

Quantitative immunohistochemical evaluation revealed a statistically significant increase in the Ki-67 proliferation index in diabetic patients compared to controls. In hyperkeratotic and verrucous lesions, mean Ki-67 expression exceeded 34.7% ± 6.1% of basal and suprabasal keratinocytes in the diabetic cohort versus 18.2% ± 4.9% in non-diabetic cases ($U = 132.5$, $p < 0.001$). Nuclear p53 accumulation, indicative of disrupted cell cycle regulation and genomic stress, was identified in 35 of 40 diabetic patients (87.5%), compared to 12 of 28 in the control group (42.8%; $\chi^2 = 14.9$, $p = 0.003$). The mean H-score for MMP-9, reflecting enzymatic degradation potential within the basal lamina and extracellular matrix, reached 186.4 ± 23.5 in the diabetic subgroup and 104.2 ± 18.1 in non-diabetics ($p < 0.001$). Spearman correlation analysis confirmed a strong association between MMP-9 expression and epithelial dysplasia grade ($\rho = 0.68$, $p = 0.006$).

Microbiota analysis based on 16S rRNA sequencing revealed a dysbiotic shift in the diabetic subgroup characterized by a statistically significant increase in the relative abundance of Gram-negative anaerobes. Fusobacterium nucleatum represented 9.6% ± 2.3% of total bacterial reads in diabetic samples compared to 3.1% ± 1.7% in controls ($p = 0.008$). Similarly, Porphyromonas gingivalis exhibited elevated abundance in the diabetic cohort (5.7% ± 1.9% vs. 2.3% ± 1.2%; $p = 0.015$). The mycobiome component Candida albicans was identified via qPCR in 70.0% of diabetic cases (mean ITS copy number: 4.2×10^4) versus 32.1% of controls (1.3×10^4; $p = 0.002$). These microbial patterns were consistently associated with high-risk histopathological features, particularly in patients with erosive or verrucous leukoplakia phenotypes.

Data integration revealed a coordinated pattern linking epithelial proliferation, loss of tumor suppressor activity, and matrix degradation with increased colonization by virulence-associated microorganisms. Table 1 presents the comparative quantitative parameters across the two study groups.

Table 1.

Quantitative associations between immunohistochemical markers and microbial taxa in patients with oral leukoplakia (n = 68)

Parameter	Diabetic patients (n = 40)	Non-diabetic patients (n = 28)	p-value
Ki-67 proliferation index (%)	34.7 ± 6.1	18.2 ± 4.9	< 0.001

p53-positive nuclei (%)	87.5	42.8	0.003
MMP-9 H-score	186.4 ± 23.5	104.2 ± 18.1	< 0.001
F. nucleatum (% relative reads)	9.6 ± 2.3	3.1 ± 1.7	0.008
P. gingivalis (% relative reads)	5.7 ± 1.9	2.3 ± 1.2	0.015
C. albicans detection rate (%)	70.0	32.1	0.002
C. albicans copy number ($\times 10^4$)	4.2	1.3	0.002

The convergence of molecular dysregulation and microbiota expansion suggests the formation of a pro-oncogenic mucosal microenvironment in patients with impaired carbohydrate metabolism. The combined elevation of proliferative markers, p53 dysregulation, and increased matrix metalloproteinase activity in the context of microbial virulence underscores the pathogenic role of dysbiosis in accelerating leukoplakia progression. The predominance of F. nucleatum and P. gingivalis correlates with epithelial barrier disruption and nuclear instability, consistent with patterns observed in other inflammation-driven carcinogenic models. Fungal overgrowth, particularly by C. albicans, further amplifies inflammatory signaling and may contribute to epithelial–mesenchymal transition in predisposed tissues.

These findings reinforce the necessity of incorporating microbial surveillance and molecular stratification into the clinical management algorithm of oral leukoplakia, particularly in patients with metabolic comorbidities. Microbiota-informed risk profiling may enable earlier identification of transformation-prone lesions and support precision-targeted interventions.

CONCLUSION

The present study establishes a clinically and statistically substantiated association between metabolic dysregulation and microbial shifts in the oral mucosa of patients with leukoplakia. The data demonstrate that carbohydrate metabolism disorders, particularly type 2 diabetes mellitus, significantly exacerbate epithelial proliferation, tumor suppressor pathway disruption, and extracellular matrix degradation. These molecular alterations co-occur with increased colonization by high-risk microbial taxa, including Fusobacterium nucleatum, Porphyromonas gingivalis, and Candida albicans. The spatial and quantitative correlation between dysbiosis and dysplastic transformation supports the hypothesis of microbiome-driven modulation of leukoplakia progression.

Microbiota profiling, in conjunction with immunohistochemical risk markers (Ki-67, p53, MMP-9), offers a promising framework for stratifying malignant potential in precancerous lesions. Future diagnostic algorithms should integrate microbial and molecular data to improve risk prediction and to guide targeted surveillance in patients with systemic metabolic impairments. The findings further justify the inclusion of microbiome modulation strategies as adjunctive measures in the clinical management of high-risk leukoplakia.

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