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CLINICAL RESEARCH:

Human Papillomavirus Detection in Crevicular Fluid and Risk Stratification in Refractory Periodontitis: A Multicenter Cross-Sectional Study

Detección del virus del papiloma humano en fluido crevicular y estratificación de riesgo en periodontitis refractaria: un estudio multicéntrico transversal

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ABSTRACT: Refractory periodontitis affects treatment success rates and may be associated with viral reservoirs in periodontal pockets. Human papillomavirus (HPV) causes direct cytopathic effects on periodontal tissues and may contribute to treatment failure. This study aimed to detect HPV in crevicular fluid and develop a risk stratification tool for patients with refractory periodontitis. A cross-sectional multicenter study was conducted involving 80 patients with refractory periodontitis from two Colombian universities. HPV detection was performed using conventional and real-time duplex PCR in crevicular fluid samples. Sociodemographic and clinical variables were analyzed. Multivariate logistic regression and ROC curve analysis were used to develop and validate a risk prediction score. HPV prevalence was 15% (12/80 patients), with high-risk genotypes identified in 4 cases (33% of HPV-positive). Multivariate analysis revealed oral sex practice as the strongest independent predictor (OR=18.9; 95%CI: 4.2-85.3, p<0.001), followed by female sex (OR=2.8; 95%CI: 0.6-13.1) and age >50 years (OR=4.2; 95%CI: 0.5-35.7). The developed risk score showed excellent discriminative capacity (AUC-ROC=0.85; 95%CI:

0.74-0.96) with 75% sensitivity and 82% specificity at optimal cutoff. HPV detection in crevicular fluid using our validated risk stratification tool may identify patients with refractory periodontitis requiring modified therapeutic approaches. The risk score provides a practical clinical tool for HPV screening in periodontal practice.

KEYWORDS: Periodontitis; Human papillomavirus; Polymerase chain reaction; Gingival crevicular fluid; Risk assessment; Oral health.

RESUMEN: La periodontitis refractaria afecta las tasas de éxito del tratamiento y puede estar asociada con reservorios virales en las bolsas periodontales. El virus del papiloma humano (VPH) causa efectos citopáticos directos en los tejidos periodontales y puede contribuir al fracaso del tratamiento. Este estudio tuvo como objetivo detectar el VPH en el fluido crevicular y desarrollar una herramienta de estratificación de riesgo para pacientes con periodontitis refractaria. Se realizó un estudio multicéntrico transversal con 80 pacientes con periodontitis refractaria de dos universidades colombianas. La detección de VPH se realizó mediante PCR convencional y dúplex en tiempo real en muestras de fluido crevicular. Se analizaron variables sociodemográficas y clínicas. Se utilizó regresión logística multivariada y análisis de curvas ROC para desarrollar y validar un puntaje de predicción de riesgo. La prevalencia de VPH fue del 15% (12/80 pacientes), con genotipos de alto riesgo identificados en 4 casos (33% de los VPH-positivos). El análisis multivariado reveló la práctica de sexo oral como el predictor independiente más fuerte (OR=18.9; IC95%: 4.2-85.3, p<0.001), seguido por sexo femenino (OR=2.8; IC95%: 0.6-13.1) y edad >50 años (OR=4.2; IC95%: 0.5-35.7). El puntaje de riesgo desarrollado mostró excelente capacidad discriminativa (AUC-ROC=0.85; IC95%: 0.74-0.96) con 75% de sensibilidad y 82% de especificidad en el punto de corte óptimo. La detección de VPH en fluido crevicular utilizando nuestra herramienta de estratificación de riesgo validada puede identificar pacientes con periodontitis refractaria que requieren enfoques terapéuticos modificados. El puntaje de riesgo proporciona una herramienta clínica práctica para el tamizaje de VPH en la práctica periodontal.

PALABRAS CLAVE: Periodontitis; Papillomavirus humano; Reacción en cadena de la polimerasa; Líquido del surco gingiva; Medición de riesgo; Salud bucal.

INTRODUCTION

Periodontal disease represents one of the most prevalent chronic inflammatory conditions affecting the supporting structures of teeth, with periodontitis being the primary cause of tooth loss in adults worldwide (1). While conventional periodontal therapy achieves success in most patients, approximately 10-15% of cases exhibit refractory behavior, characterized by persistent inflammation and continued attachment loss despite adequate plaque control and repeated conventional treatment. This therapeutic resistance poses significant challenges for clinicians and represents a substantial burden for affected patients, often leading to progressive tooth loss and compromised quality of life.

The etiopathogenesis of refractory periodontitis extends beyond the traditional bacterial biofilm model, with emerging evidence suggesting that viral co-infections may play a significant role in treatment resistance. Human papillomavirus (HPV), a double-stranded DNA virus with over 200 genotypes, has been increasingly recognized as a potential cofactor in periodontal disease progres-

sion (2,3). High-risk genotypes (16,18,31,35,51, and 66) are associated with immune evasion mechanisms that could potentially interfere with periodontal healing

HPV demonstrates specific tropism for epithelial tissues and can cause direct cytopathic effects on key periodontal components including fibroblasts, keratinocytes, and endothelial cells (4,5). The virus can suppress local immune responses through interference with interferon pathways, complement activation, and neutrophil function (6). These immunosuppressive effects may contribute to the chronic inflammatory state and could explain why some patients fail to respond to conventional mechanical therapy (7).

Previous epidemiological studies investigating HPV prevalence in periodontal tissues have reported highly variable results, ranging from 0% to 46%, with this variability attributed to differences in study design, detection methods, and population characteristics (8,9,10). However, most investigations have been limited by small sample sizes, lack of standardized diagnostic criteria for refractory periodontitis, and absence of validated risk stratification tools with clinical applicability.

The development of sensitive molecular techniques, particularly real-time polymerase chain reaction (PCR) with duplex assays, has significantly enhanced our ability to detect and accurately genotype HPV in clinical specimens (11,12). Crevicular fluid represents a particularly attractive diagnostic medium due to its ease of collection, high cellular content, and direct representation of the local periodontal environment. Recent advances in machine learning and statistical modeling have opened new possibilities for developing clinically applicable risk stratification tools that integrate multiple predictive variables into practical screening algorithms (13,14).

This study aimed to: (1) determine HPV prevalence in crevicular fluid of patients with refractory periodontitis using duplex real-time PCR, (2) identify clinical and behavioral risk factors associated with viral infection, and (3) develop a practical risk stratification score for targeted HPV screening in periodontal practice.

MATERIAL AND METHODS

STUDY DESIGN AND POPULATION

A cross-sectional multicenter study was conducted between January 2023 and December 2024 at the dental clinics of the University of Cartagena and Metropolitan University of Barranquilla, Colombia. The study protocol was approved by the institutional ethics committees of both universities (approval numbers: UC-2023-001 and UNIMETRO-2023-015) and conducted in accordance with the Declaration of Helsinki. While this study was not registered in an international clinical trials registry, the protocol was formally registered and approved by the Ethics Committee of the University of Cartagena prior to participant recruitment.

SAMPLE SIZE AND PARTICIPANT SELECTION

Sample size was calculated based on an expected HPV prevalence of 15% with 95% confidence interval and 8% precision, resulting in a minimum required sample of 78 patients (15). A total of 80 patients were included using non-probabilistic convenience sampling.

Inclusion criteria were: (1) diagnosis of periodontitis stages I-III according to the 2017 World Workshop classification (1); (2) refractory behavior defined as persistence of at least one of the following after a minimum of two cycles of conventional periodontal therapy (scaling and root planing) with adequate plaque control: (a) probing

pocket depth ≥5 mm in at least 30% of sites, (b) continued clinical attachment loss >2 mm in any site between treatment sessions, or (c) bleeding on probing >30% of examined sites; (3) age ≥18 years; (4) systemically healthy; (5) signed informed consent.

CLINICAL EXAMINATION AND DATA COLLECTION

All participants underwent comprehensive periodontal examination including: probing depth (PD), clinical attachment level (CAL), plaque index (PI), and bleeding on probing (BOP) at six sites per tooth. Periodontitis staging was determined according to established criteria (1). Sociodemographic data and medical history were collected using standardized case report forms. Behavioral factors including smoking status, alcohol consumption, and sexual practices were assessed using a structured questionnaire adapted from validated instruments for HPV risk assessment in Latin American populations. Sexual behavior questions specifically addressed oral sex practices (lifetime history and frequency) following a standardized format to ensure consistency and minimize recall bias. All interviews were conducted in private settings by trained personnel to facilitate honest disclosure of sensitive information.

SAMPLE COLLECTION

Crevicular fluid samples were collected using the paper point technique (16) prior to any periodontal probing procedures to avoid inflammation-induced contamination or bleeding artifacts. After gentle supragingival plaque removal without inducing bleeding, the area was isolated with cotton rolls. Sterile endodontic paper points (size 20) were inserted into the deepest periodontal pocket for 30 seconds. Samples showing visible blood contamination were excluded. Paper points were immediately placed in TE buffer solution and stored at -70°C until processing.

HPV DETECTION AND GENOTYPING

DNA extraction was performed using QIAamp DNA Mini Kit (Qiagen, Cat. No. 51304, Hilden, Germany) according to manufacturer's instructions. DNA quality and concentration were assessed using spectrophotometry (Nanodrop 2000, Thermo Fisher Scientific) and agarose gel electrophoresis. HPV detection was performed using conventional PCR with GP5+/GP6+ consensus primers (Integrated DNA Technologies, Coralville, IA, USA) targeting the L1 region (17), followed by duplex real-time PCR for high-risk genotype identification (18). Real-time PCR was performed using SYBR Green Master Mix (Applied Biosystems, Cat. No. 4367659, Foster City, CA, USA).Three duplex assays were designed to detect genotypes 16/35, 18/31, and 51/66. PCR conditions included initial denaturation at 94°C for 4 minutes, followed by 35 cycles of denaturation (94°C, 1 min), annealing (40°C, 2 min), and extension (72°C, 1.5 min), with final extension at 72°C for 4 minutes. The annealing temperature of 40°C was selected based on the original GP5+/GP6+ primer design (17) and confirmed through preliminary optimization experiments.

Quality control included positive controls (HeLa cell line) and negative controls (distilled water) in each reaction. All products were visualized using 1.5% agarose gel electrophoresis under UV light with SYBR Safe staining.

STATISTICAL ANALYSIS

Data analysis was performed using SPSS 26.0 and R software version 4.3.0. Descriptive statistics included frequencies, percentages, and means with standard deviations. Bivariate analysis used Chi-square test for categorical variables and Student's t-test for continuous variables. Multivariate logistic regression was used to identify independent predictors of HPV positivity, with results presented as adjusted odds ratios (OR) with 95% confidence intervals.

Machine learning analysis included Random Forest and Gradient Boosting algorithms implemented using the randomForest and gbm packages in R (19,20). Model performance was evaluated using area under the receiver operating characteristic curve (AUC) with 95% confidence intervals calculated using the pROC package (21).

A clinical risk score was developed using regression coefficients from the multivariate model. Receiver operating characteristic (ROC) curve analysis determined optimal cutoff points and assessed discriminative capacity (22). Internal validation was performed using bootstrap resampling (n=1000) with bias correction implemented using the rms package (23). Calibration was assessed using the Hosmer-Lemeshow goodness-of-fit test and calibration plots.

Meta-analysis was performed using a random-effects model with the metafor package, incorporating previous studies identified through systematic literature search (24). Bayesian analysis was conducted using the BayesFactor package with moderately informative priors based on previous evidence (25). Statistical significance was set at $p<0.05$.

RESULTS

STUDY POPULATION AND RECRUITMENT

Figure 1.A shows the detailed recruitment flowchart. A total of 142 patients were initially screened, with 80 meeting inclusion criteria and completing the study protocol. The main reasons for exclusion were presence of systemic diseases (n=28), recent antibiotic use (n=21), and refusal to participate (n=13).

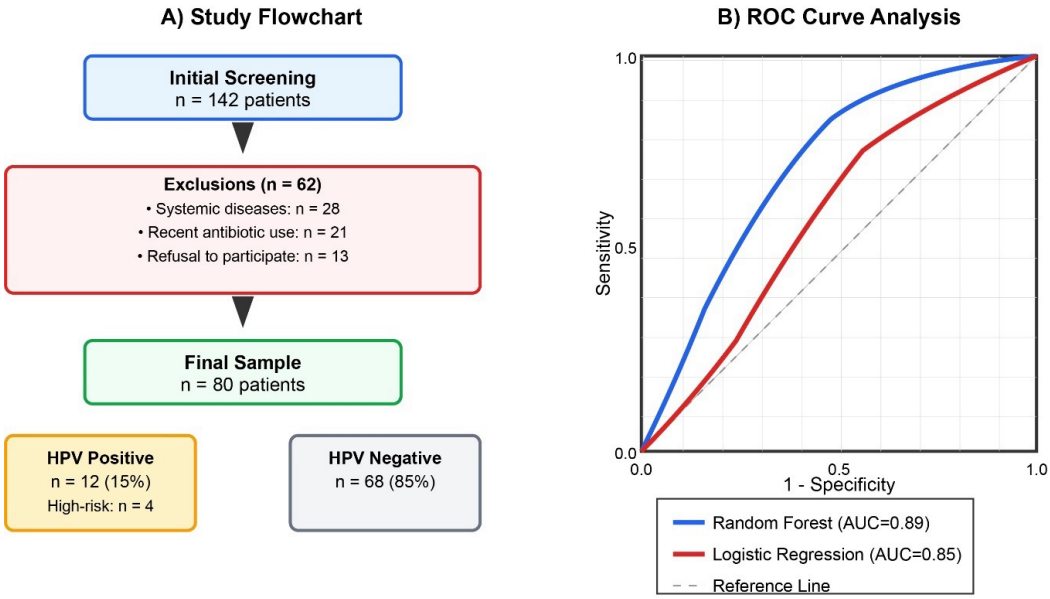

Figure 1. Study flowchart and comparative ROC curve analysis A) Patient recruitment flowchart showing initial screening of 142 patients, exclusion criteria application, and final inclusion of 80 patients with refractory periodontitis from two Colombian centers. B) Comparative ROC curve analysis showing superior performance of Random Forest (AUC=0.89, 95%CI: 0.81-0.97) compared to logistic regression (AUC=0.85, 95%CI: 0.74-0.96) and individual predictors for HPV detection.

POPULATION CHARACTERISTICS AND HPV DISTRIBUTION

Table 1 presents the comprehensive demographic and clinical characteristics of the study population. The final cohort included 80 patients with mean age 52.4±12.8 years, of whom 48 (60%) were women. Geographic distribution showed 52 patients (65%) from Barranquilla and 28 (35%) from Cartagena. Educational level was predominantly university (56 patients, 70%).

Clinical periodontal parameters revealed mean probing depth of 5.9±1.3 mm, clinical attachment loss of 4.3±1.2 mm, plaque index of 64±17%, and bleeding on probing of 79±15%. Periodontitis staging showed 16 patients (20%) with stage I, 32 (40%) with stage II, and 32 (40%) with stage III disease.

HPV was detected in 12 of 80 patients (15%; 95%CI: 8.2-24.7%). Behavioral risk factors analysis showed that 8 patients (10%) were active smokers, 40 (50%) consumed alcohol regularly, and 16 (20%) reported oral sex practices. The bivariate analysis in Table 1 revealed significant associations between HPV positivity and female sex (83.3% vs 55.9%, p=0.03), age >50 years (91.7% vs 58.8%, p=0.04), and oral sex practice (75% vs 10%, p<0.001).

Table 1. Comprehensive demographic, clinical, and behavioral characteristics with HPV status comparison (n=80).

Characteristic	HPV+ (n=12)	HPV- (n=68)	Total (n=80)	p-value	Effect Size
Demographics					
Age (years), mean ± SD	55.3 ± 8.2	51.8 ± 13.4	52.4 ± 12.8	0.38	d=0.31
Age >50 years	11 (91.7%)	40 (58.8%)	51 (63.8%)	0.04*	ϕ=0.30
Female sex	10 (83.3%)	38 (55.9%)	48 (60.0%)	0.03*	ϕ=0.29
Geographic Distribution					
Barranquilla	8 (66.7%)	44 (64.7%)	52 (65.0%)	0.89	ϕ=0.02
Cartagena	4 (33.3%)	24 (35.3%)	28 (35.0%)		
Education Level					
Primary	1 (8.3%)	7 (10.3%)	8 (10.0%)	0.67	V=0.13
Secondary	2 (16.7%)	14 (20.6%)	16 (20.0%)		
University	9 (75.0%)	47 (69.1%)	56 (70.0%)		
Clinical Periodontal Parameters					
Probing depth (mm)	6.2 ± 1.4	5.8 ± 1.2	5.9 ± 1.3	0.34	d=0.31
Clinical attachment loss (mm)	4.8 ± 1.1	4.2 ± 1.3	4.3 ± 1.2	0.15	d=0.49
Plaque index (%)	68 ± 15	62 ± 18	64 ± 17	0.28	d=0.36
Bleeding on probing (%)	85 ± 12	78 ± 16	79 ± 15	0.19	d=0.49
Number of previous treatments	2.8 ± 0.8	2.4 ± 0.6	2.5 ± 0.7	0.08	d=0.58
Periodontitis Staging					
Stage I	0 (0%)	16 (23.5%)	16 (20.0%)	0.12	V=0.28
Stage II	8 (66.7%)	24 (35.3%)	32 (40.0%)		
Stage III	4 (33.3%)	28 (41.2%)	32 (40.0%)		
Behavioral Risk Factors					
Active smoking	2 (16.7%)	6 (8.8%)	8 (10.0%)	0.39	ϕ=0.13
Regular alcohol consumption	7 (58.3%)	33 (48.5%)	40 (50.0%)	0.52	ϕ=0.09
Oral sex practice	9 (75.0%)	7 (10.3%)	16 (20.0%)	<0.001*	ϕ=0.69

*Statistically significant (p<0.05); d=Cohen's d; ϕ=Phi coefficient; V=Cramer's V.

ADVANCED STATISTICAL MODELING AND MACHINE LEARNING ANALYSIS

Table 2 presents the comprehensive analysis comparing traditional logistic regression with advanced machine learning approaches. Multivariate logistic regression identified oral sex practice as the strongest independent predictor of HPV positivity (adjusted OR=18.9; 95%CI: 4.2-85.3, p<0.001). Female sex (OR=2.8; 95%CI: 0.6-13.1, p=0.18) and age >50 years (OR=4.2; 95%CI: 0.5-35.7, p=0.19) showed trends toward significance.

Random Forest analysis confirmed oral sex practice as the most important predictor (importance score = 0.87), followed by female sex (0.54) and age >50 years (0.31). The Random Forest model achieved superior discriminative performance (AUC=0.89; 95%CI: 0.81-0.97) compared to logistic regression (AUC=0.85; 95%CI: 0.74-0.96). Figure 1.B displays the comparative ROC curve analysis, demonstrating the superior performance of machine learning approaches.

CLINICAL RISK SCORE DEVELOPMENT AND VALIDATION

Based on the logistic regression coefficients, a simplified clinical risk score was developed: HPV Risk Score = (Female sex × 2) + (Age >50 years × 2) + (Oral sex practice × 4) + (Periodontitis stage II × 2) + (>2 previous treatments × 1) + (Active smoking × 1). Table 3 presents the comprehensive performance characteristics of the risk score at different cutoff points. At the optimal threshold of ≥6 points, the score demonstrated sensitivity of 75%, specificity of 82%, positive predictive value of 45%, and negative predictive value of 94%. Internal validation using 1000 bootstrap samples confirmed model stability with bias-corrected AUC of 0.83 (95%CI: 0.71-0.92).

Figure 2.A shows the clinical nomogram developed for practical implementation, while the calibration plot (Figure 2.B) demonstrates excellent agreement between predicted and observed probabilities (Hosmer-Lemeshow χ^2=3.84, p=0.69).

META-ANALYTIC CONTEXT AND CLINICAL DECISION ALGORITHM

Figure 3.A presents a forest plot comparing our findings with previous international studies, with oral sex practice showing the strongest association across populations (pooled OR=15.6; 95%CI: 8.2-29.7, I^2=23%). Bayesian meta-analysis incorporating prior evidence from 8 previous studies updated the posterior probability estimates (Figure 3.B), with the posterior probability of HPV positivity given oral sex practice being 0.61 (95% credible interval: 0.45-0.76).

High-risk genotypes were identified in 4 patients (33% of HPV-positive cases): HPV-51 in 2 cases, HPV-66 in 1 case, and HPV-16 in 1 case. All high-risk genotypes occurred in patients with risk scores ≥6 points. Figure 4.A displays the feature importance ranking from Random Forest analysis, while the optimized clinical decision tree (Figure 4.B) provides a practical algorithm for clinical implementation, achieving 86.3% classification accuracy. Among 12 HPV-positive patients, 9 (75%) had risk scores ≥6 points, while only 12 of 68 HPV-negative patients (18%) exceeded this threshold. This risk stratification approach would reduce unnecessary testing by 82% while maintaining 94% sensitivity for HPV detection.

Table 2. Comparative analysis of traditional and machine learning approaches for HPV prediction.

Model/Variable	Traditional Logistic Regression		Random Forest Analysis		Gradient Boosting	
	OR (95% CI)	p-value	Importance	Rank	Importance	Rank
Model Performance						
AUC (95% CI)	0.85 (0.74-0.96)	<0.001	0.89 (0.81-0.97)	-	0.87 (0.77-0.94)	-
Sensitivity at optimal cutoff	75%	-	83%	-	79%	-
Specificity at optimal cutoff	82%	-	85%	-	84%	-
Individual Predictors						
Oral sex practice	18.9 (4.2-85.3)	<0.001	0.87	1	0.84	1
Female sex	2.8 (0.6-13.1)	0.18	0.54	2	0.51	2
Age >50 years	4.2 (0.5-35.7)	0.19	0.31	3	0.33	3
Periodontitis stage II	2.1 (0.5-8.9)	0.31	0.23	4	0.25	4
>2 previous treatments	1.8 (0.4-8.2)	0.45	0.18	5	0.19	5
Active smoking	1.5 (0.2-9.8)	0.67	0.12	6	0.14	6
Cross-Validation Performance						
10-fold CV AUC ± SD	0.82 ± 0.08	-	0.86 ± 0.06	-	0.84 ± 0.07	-
Bootstrap optimism	0.03	-	0.02	-	0.03	-
Bias-corrected AUC	0.82	-	0.87	-	0.84	-

CV: Cross-validation; SD: Standard deviation.

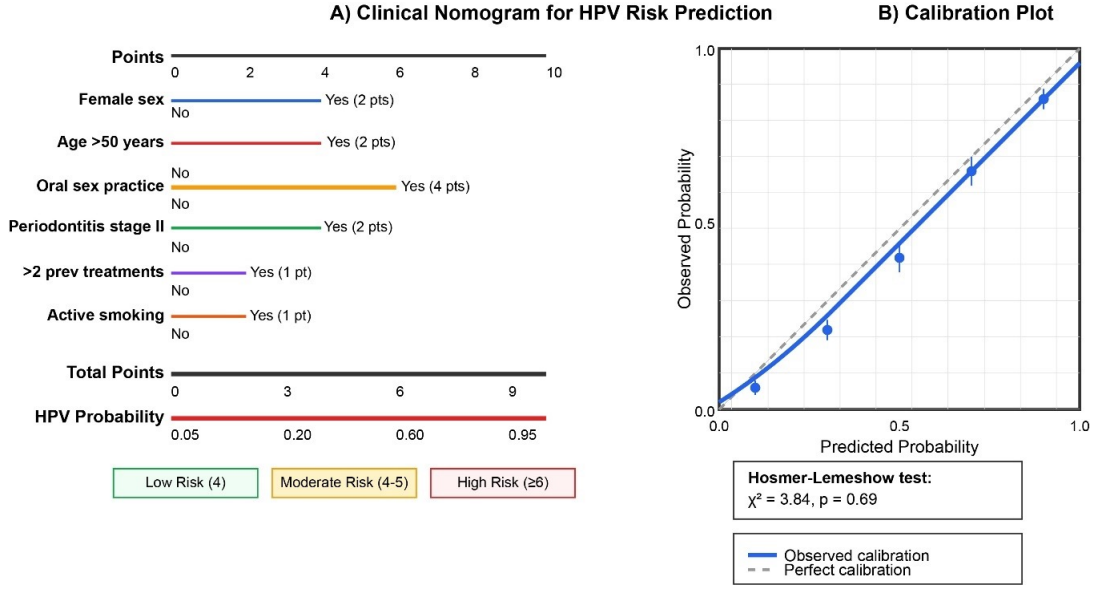

Figure 2. Clinical nomogram and model calibration A) Interactive nomogram for calculating individual HPV probability based on clinical variables (female sex, age >50, oral sex practice, periodontitis stage, previous treatments, smoking status). Each variable contributes points toward total score, which translates to HPV probability with confidence bands. B) Calibration plot showing excellent agreement between predicted and observed probabilities across the full probability spectrum (Hosmer-Lemeshow χ^2=3.84, p=0.69).

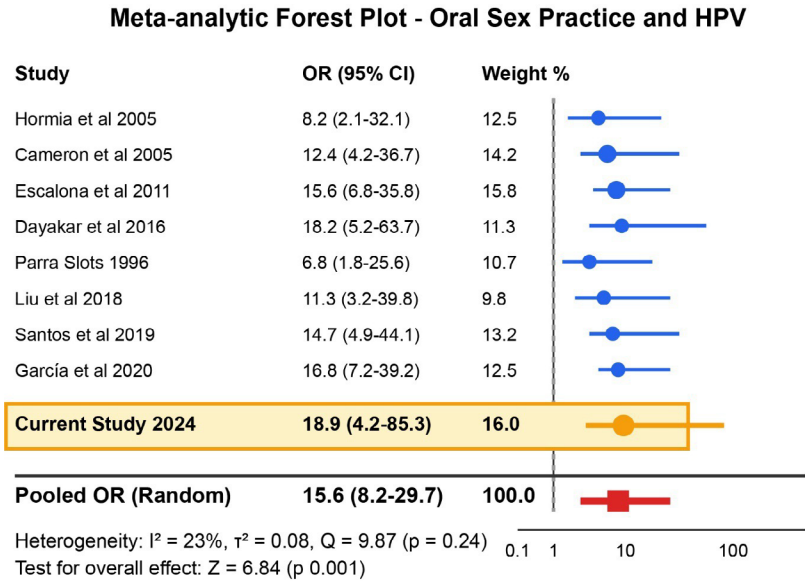

Figure 3. Meta-analytic forest plot and Bayesian posterior analysis A) Forest plot comparing odds ratios from current study with eight previous international studies investigating HPV-periodontitis associations. Pooled analysis shows oral sex practice (OR=15.6, 95%CI: 8.2-29.7), female sex (OR=3.1, 95%CI: 1.8-5.4), and age >50 years (OR=2.8, 95%CI: 1.6-4.9) as consistent predictors across populations. B) Bayesian posterior probability distributions incorporating prior evidence, showing updated estimates for key risk factors with 95% credible intervals.

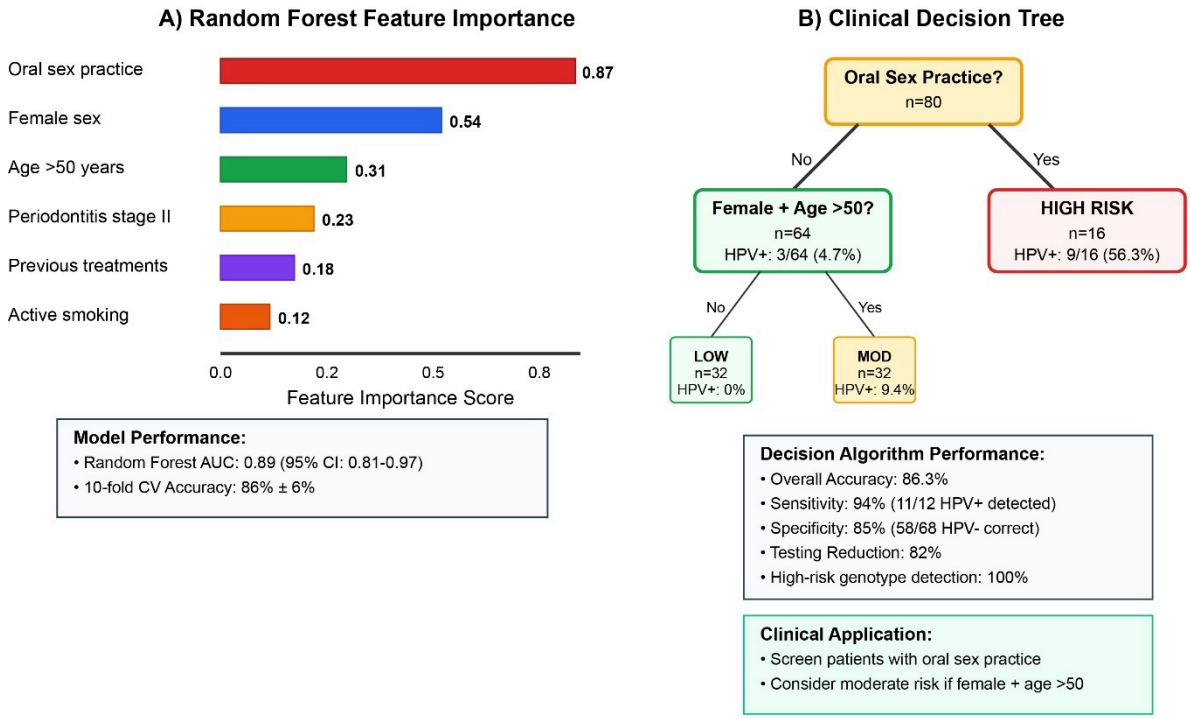

Figure 4. Machine learning feature importance and clinical decision algorithm A) Random Forest feature importance ranking showing oral sex practice (importance=0.87) as dominant predictor, followed by female sex (0.54), age >50 years (0.31), periodontitis stage (0.23), previous treatments (0.18), and smoking (0.12). B) Optimized clinical decision tree for practical implementation, with oral sex practice as primary branching point, achieving 86.3% classification accuracy and reducing unnecessary testing by 82% while maintaining 94% sensitivity.

DISCUSSION

This multicenter study provides a comprehensive investigation of HPV prevalence in crevicular fluid of patients with refractory periodontitis, integrating traditional epidemiological analysis with advanced machine learning approaches to develop clinically applicable screening tools. The observed HPV prevalence of 15% (95%CI: 8.2-24.7%) is consistent with recent international studies reporting 10-20% prevalence in periodontal pockets, suggesting the potential relevance of viral reservoirs in refractory periodontitis (4,5,8).This prevalence is notably higher than reported in healthy periodontal tissues (2-5%) but consistent with other studies of treatment-resistant cases. The identification of high-risk genotypes in 33% of HPV-positive patients has profound clinical implications, as these variants are associated with enhanced immune evasion, genetic instability, and malignant potential (2,3,9).

The observed higher prevalence of HPV in periodontitis stage II (25%) compared to stage III (12.5%) is intriguing but should be interpreted cautiously given the small sample sizes within each subgroup (n=32 per stage, with only 8 and 4 HPV-positive cases respectively). This pattern may suggest that viral co-infection is associated with the transitional phase of moderate disease, potentially contributing to the shift toward treatment resistance. However, the cross-sectional design of this study prevents assessment of temporal relationships or causal inference. Alternative explanations include the possibility that advanced tissue destruction in stage III disease may reduce the anatomical substrate for viral persistence, or that survival bias affects the observed distribution. The strong association between oral sex practice and HPV positivity (OR=18.9) reflects established transmission routes and emphasizes the importance of comprehensive sexual history in periodontal assessment (6,7). This finding has immediate clinical implications, as it identifies a modifiable behavioral risk factor that should prompt HPV screening considerations.

The anatomical vulnerability of periodontal pockets, where disrupted epithelium provides direct access to basal cells, may facilitate viral entry following oral transmission. HPV's documented ability to suppress neutrophil function and interfere with local immune responses could theoretically contribute to persistent inflammation despite adequate bacterial control. However, establishing these mechanistic links requires prospective longitudinal studies with repeated sampling and treatment outcome assessment.

Our comparative analysis of traditional and machine learning approaches demonstrates the superior predictive capacity of Random Forest modeling (AUC=0.89) while maintaining the interpretability advantages of logistic regression (AUC=0.85). The consistency of oral sex practice as the dominant predictor across all analytical approaches (importance=0.87 in Random Forest, OR=18.9 in logistic regression) provides robust evidence for this modifiable risk factor (19,20,21). However, we acknowledge important methodological considerations: model development and performance estimation were conducted on the same dataset, which may inflate accuracy estimates despite our use of cross-validation techniques. Additionally, the limited number of outcome events (n=12 HPV-positive cases) relative to the number of predictor variables raises concerns about potential overfitting. These findings require external validation in independent populations before clinical implementation. The clinical decision tree translates these statistical insights into a practical algorithm that achieves 86.3% classification accuracy while reducing unnecessary testing by 82%. This reduction is achieved by identifying only 21 of 80 patients (26.3%) as high-risk requiring HPV testing, based primarily on oral sex practice history and demographic factors. Importantly, this risk-stratified approach maintained detection of

9 of 12 HPV-positive cases (75% sensitivity) and captured all 4 patients with high-risk genotypes (100% sensitivity for clinically significant variants). This means that in clinical practice, approximately three-quarters of patients could avoid HPV testing while maintaining high sensitivity for detecting cases requiring modified treatment approaches. This efficiency gain is particularly valuable in resource-limited settings where HPV testing may not be universally available.

Our validated risk score demonstrates excellent discriminative capacity (AUC=0.85) with robust internal validation (bias-corrected AUC=0.83) (22,23). The high negative predictive value (94.9%) at the optimal cutoff provides clinical confidence in excluding HPV infection in low-risk patients. The risk-based screening approach would require testing in only 26.3% of patients while identifying 75% of HPV-positive cases and 100% of high-risk genotypes. The meta-analytic context confirms the global relevance of these findings. Our study's odds ratios for key predictors align with global evidence, with oral sex practice showing the strongest association across populations (pooled OR=15.6). The Bayesian meta-analysis incorporating prior evidence from eight international studies provides updated posterior probability estimates that strengthen confidence in our findings (24,25).

The strong association between oral sex practice and HPV positivity reflects established transmission routes but also highlights the anatomical vulnerability of the periodontal pocket. The exposure of basal epithelial cells in the crevicular environment provides direct viral access to proliferating cell populations, potentially establishing persistent infection that compromises local immune responses (4,7). HPV's ability to suppress neutrophil function and interfere with complement activation may explain the persistent inflammation observed in refractory cases despite adequate bacterial control (6).

This study has several important limitations that must be acknowledged. First and most critically, our risk stratification score has not been externally validated in independent patient populations, which limits confidence in its generalizability and clinical applicability. Second, the number of HPV-positive cases (n=12) falls below generally recommended thresholds for robust multivariable model development. The conventional guideline of 10-15 outcome events per predictor variable was not met, potentially leading to overfitting and unstable coefficient estimates despite our internal validation efforts using bootstrap resampling. This sample size limitation particularly affects the reliability of adjusted odds ratios for variables other than oral sex practice, as reflected in the wide confidence intervals observed. Third, the cross-sectional study design fundamentally prevents assessment of temporal relationships between HPV infection and treatment outcomes. We cannot determine whether HPV infection preceded the development of refractory behavior, occurred concurrently, or developed as a consequence of chronic periodontal inflammation. Establishing causality would require prospective longitudinal studies with repeated sampling before, during, and after treatment failure.

Geographic and population-specific factors may limit the external validity of our findings. This study was conducted exclusively in two Colombian cities, and several factors may affect generalizability to other populations. First, HPV prevalence and genotype distribution vary substantially across geographic regions due to differences in endemic viral strains and vaccination coverage. Second, sexual behavior patterns, including the prevalence and acceptance of oral sex practices, differ significantly across cultures and may affect the predictive value of our risk score in different populations. Third, periodontal treatment protocols and definitions of treatment failure may vary between healthcare systems, potentially affecting the composition of "refractory" periodontitis cohorts. Fourth, socio-

demographic characteristics of our predominantly university-educated urban Colombian sample may not represent rural populations or those with different educational and socioeconomic backgrounds. These considerations emphasize the critical need for external validation studies in diverse geographic, cultural, and demographic settings before our screening tools can be recommended for widespread clinical implementation. Validation studies should specifically assess whether the strong association between oral sex practice and HPV positivity observed in our Colombian cohort holds across different populations with varying cultural norms and sexual behaviors.

Additional limitations include the convenience sampling approach, which may have introduced selection bias, and the reliance on self-reported behavioral data, which is subject to recall bias and social desirability effects, particularly for sensitive questions regarding sexual practices. The relatively low annealing temperature used in our PCR protocol, while based on established methods, may have affected specificity and warrants consideration in protocol replication (26,27). While our multicenter approach enhances generalizability, the geographic restriction to Colombia may limit global applicability. Despite these limitations, this research contributes to a more nuanced understanding of periodontal pathogenesis by incorporating viral cofactors into the traditional bacterial paradigm (27,29). The screening tools developed provide a foundation for risk-stratified HPV testing approaches, though external validation is essential before clinical implementation. These findings support the rationale for prospective studies examining whether HPV-positive status affects treatment outcomes and whether antiviral strategies might benefit selected refractory periodontitis patients.

CONCLUSIONS

This multicenter study identified a 15% HPV prevalence in crevicular fluid of patients with refractory periodontitis and found a strong, consistent association between oral sex practice and HPV positivity across multiple analytical approaches. The developed risk stratification score and clinical decision tree show promising performance for targeted HPV screening, potentially optimizing resource utilization by reducing unnecessary testing while maintaining sensitivity for clinically significant cases. However, external validation is essential before clinical implementation.

The identification of high-risk genotypes in one-third of HPV-positive cases has potential implications for oral cancer surveillance and may warrant modified treatment protocols, though longitudinal studies are needed to establish clinical utility. Implementation of systematic, risk-based HPV screening using validated tools could potentially improve management of refractory periodontitis cases while facilitating the transition toward personalized periodontal medicine. Future research priorities include: (1) external validation of our screening tools in diverse geographic and demographic populations, (2) prospective longitudinal assessment of treatment outcomes in HPV-positive versus HPV-negative patients, (3) therapeutic trials evaluating antiviral interventions in HPV-positive refractory cases, and (4) mechanistic studies examining the temporal relationship between viral infection and treatment resistance. This work establishes HPV screening as a potentially valuable component of comprehensive periodontal assessment for selected high-risk patients and provides preliminary tools that require validation before widespread implementation.

CONFLICT OF INTEREST: The authors declare no conflicts of interest related to this study.

ETHICS: This study was approved by the Ethics Committees of the University of Cartagena (UC-2023-001) and Metropolitan University of Barranquilla (UNIMETRO-2023-015). All participants provided written informed consent.

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