Research Article

Epstein-Barr Virus Latent Membrane Protein 1 Expression Identifies a Latency II-Consistent Subset of Oral Squamous Cell Carcinoma: A Case-Control Study from Najaf, Iraq

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ABSTRACT

Background & Objectives: Although Epstein-Barr virus (EBV) is firmly implicated in nasopharyngeal carcinoma, its role in oral squamous cell carcinoma (OSCC) remains contested. LMP1, the principal EBV oncoprotein, drives cellular transformation and blocks epithelial differentiation. We assessed LMP1 immunohistochemical expression and its concordance with EBER in an Iraqi OSCC cohort, and whether the pattern is consistent with a Latency II programme.
Methods: In a retrospective case-control study, 50 OSCC cases and 50 histologically normal oral mucosa controls from Najaf were examined. EBV was localised by chromogenic EBER in situ hybridisation; LMP1 was detected by immunohistochemistry (rabbit monoclonal clone D24-G, 1:100). Non-parametric tests were applied throughout, with Cohen's κ and McNemar's test for LMP1–EBER agreement.
Results: LMP1 was detected in 13/50 cases (26.0%) and no controls (Fisher's exact P = 0.0001; Haldane–Anscombe OR = 36.36), and moderate-to-strong expression predominated among positives (8/13). A non-significant trend toward higher positivity was seen in moderately differentiated tumours (Grade 2, 55.6%, vs 21.2% and 12.5%; H = 5.230, P = 0.073), with no association with sex (P = 0.746) or age (P = 0.303). All 13 LMP1-positive cases were EBER-positive, whereas 9 of 22 EBER-positive cases lacked LMP1 (κ = 0.618; McNemar P = 0.004).
Conclusion: Active, Latency II-consistent EBV signalling characterises a defined 26% subset of Iraqi OSCC (Stratum A, LMP1+/EBER+), alongside an 18% Latency 0/I-consistent subset (Stratum B, LMP1−/EBER+). This asymmetric concordance provides molecular evidence for coexisting latency programmes in oral carcinogenesis, although sub-threshold LMP1 expression cannot be excluded for Stratum B.

Key words:
Epstein-Barr virus; Latent membrane protein 1; Oral squamous cell carcinoma; EBER in situ hybridisation; Viral latency programmes; Immunohistochemistry; Iraq.

INTRODUCTION

Oral squamous cell carcinoma (OSCC) is the most common malignancy of the oral cavity, accounting for approximately 90% of all oral cancers; worldwide, some 300,000 new cases are diagnosed annually, and OSCC ranks as the sixth most common malignancy globally [1]. Tobacco and alcohol, particularly in combination, remain the principal risk factors [1,2], and tobacco exposure has additionally been linked to EBV activation [3]. However, incidence is rising in younger people without these classical exposures, implicating additional aetiological factors in head-and-neck carcinogenesis [4]; oncogenic viruses, including HPV in oropharyngeal carcinoma and EBV, have been increasingly investigated [5,6,7].

The evidence linking Epstein-Barr virus (EBV) infection to OSCC risk remains inconsistent across regional studies [8,9,10,11], partly because of methodological differences in EBV detection [4,12]. Polymerase chain reaction is highly sensitive for EBV DNA but cannot localise the virus or distinguish EBV-driven disease from bystander infection [13]. Because more than 90% of adults harbour latent EBV, tumour-infiltrating lymphocytes may account for high PCR positivity rates and confound the true cellular origin of the amplified genome [13]; earlier studies did not exclude contamination from EBV-harbouring B lymphocytes that abundantly infiltrate oral epithelia [14]. Chromogenic in situ

hybridisation for EBERs is therefore the detection gold standard, since EBER RNA is expressed in all latency stages and is localised at the single-cell level [5,6]. We employed EBER-ISH to confirm epithelial localisation and exclude lymphocyte contamination [7].

Whether EBV acts as a driver or a passenger in oral carcinogenesis remains debated [12]. The weak detection of EBV DNA in OSCC relative to controls has been interpreted through a hit-and-run mechanism, in which viral DNA acts as an initiator and is progressively lost during malignant transformation [15,16,17]. Defining the function of EBV proteins when expressed is therefore central to establishing a causal role [7]. The latent membrane protein 1 (LMP1), the principal oncoprotein of EBV, is essential for cellular transformation but becomes dispensable once cells are transformed [18]. By mimicking constitutively active CD40, LMP1 engages NF-κB, JNK–p38, ERK–MAPK, PI3K–AKT, and JAK-STAT signalling [19,20,21], driving proliferation, anti-apoptosis, adhesion-molecule expression, and angiogenesis [20,22].

All EBV-associated epithelial cancers express a Latency II programme or a variant thereof [23,24]. Latency II, characteristic of nasopharyngeal carcinoma and Hodgkin lymphoma, comprises EBNA1, LMP1, LMP2, BARTs, and EBERs [23,25]. Critically, EBER expression occurs across all four latency programmes (0–III), whereas LMP1 is restricted to Latency II and III [25]; consequently, EBER+/LMP1+ specimens indicate Latency II-consistent signalling, whereas EBER+/LMP1− specimens are most consistent with Latency 0 or I. We therefore investigated LMP1 immunohistochemical expression and its concordance with EBER in an Iraqi OSCC cohort, and examined whether the observed pattern reflects an active Latency II programme or a more complex mixture of latency states.

METHODOLOGY

Study Cohort and Tissue Procurement

In this retrospective case-control study, 100 formalin-fixed, paraffin-embedded (FFPE) tissue blocks were examined: 50 from primary, previously untreated OSCC, and 50 from histologically normal oral mucosa of individuals without oral malignancy. Control specimens were obtained independently of the case patients and were not tumour-adjacent tissue; each was confirmed free of dysplasia and malignancy by two oral pathologists who reviewed every case. Consecutive eligible archival blocks diagnosed at the Pathology Department of Al-Sadder Medical City (Najaf, Iraq) between November 2024 and September 2025 were retrieved until 50 cases and 50 controls were obtained. Patients previously treated with chemotherapy or radiotherapy were excluded. Sample size was determined by the number of eligible, well-preserved archival cases available during the inclusion window rather than by a formal a priori power calculation, a constraint acknowledged in the limitations. All specimens were anonymised and assigned a unique code.

Chromogenic In Situ Hybridisation (EBER-ISH)

Chromogenic in situ hybridisation for EBER-1 and EBER-2 was performed using the ZytoFast EBV Probe kit (ZytoVision GmbH, Bremerhaven, Germany). After deparaffinisation and rehydration of 4-µm sections, heat-induced epitope retrieval was carried out in EDTA buffer (pH 9), followed by hybridisation with digoxigenin-labelled probes at 55°C, high-stringency washes, and detection with the NBT/BCIP chromogenic substrate. A dark blue-violet granular nuclear precipitate within epithelial cells indicated a positive reaction; the full EBER-ISH scoring scheme and inter-rater agreement for this cohort are reported in the companion EBER investigation.

Immunohistochemical Detection of LMP1

LMP1 protein was detected by immunohistochemistry. Sections (5 µm) on positively charged slides were baked at 60°C for 10 min, deparaffinised in xylene, rehydrated through graded ethanol, and subjected to heat-induced antigen retrieval in 10 mM sodium citrate buffer (pH 6.0) at 95°C for 20 min. Endogenous peroxidase was blocked with 3% H_2O_2 for 10 min. The biotin-free EXPOSE Mouse and Rabbit Specific HRP/DAB Detection kit (Abcam, Cat. No. ab80436) was used with a rabbit monoclonal anti-EBV LMP1 primary antibody (clone D24-G, Abcam Cat. No. ab136633) at 1:100, incubated overnight at 4°C. Visualisation used DAB chromogen with Mayer's haematoxylin counterstain and DPX mounting. EBV-positive Hodgkin lymphoma served as a positive control; substitution of the primary antibody with phosphate-buffered saline served as the negative control.

Morphometric Scoring and Evaluation

LMP1 was evaluated by light microscopy at ×400 and ×1000; positivity was defined as granular or membranous brown cytoplasmic and/or membranous staining of neoplastic cells. A semi-quantitative immunoreactive score combined the percentage of positive tumour cells — 0 (0%), 1 (1–25%), 2 (26–50%), 3 (>50%) — with staining intensity graded 0 (negative), 1 (weak), 2 (moderate), and 3 (strong); the score was averaged across ten high-power fields per case. Two experienced oral pathologists, blinded to the clinical and viral status of each case, scored all specimens independently and reached consensus for any discordant scores; inter-rater agreement before consensus was substantial (Cohen's κ = 0.84 for LMP1 positivity).

Biostatistical Analysis

Analyses were performed in IBM SPSS Statistics v28.0 (IBM Corp., Armonk, NY, USA) and independently verified in Python SciPy v1.11; a two-tailed $P < 0.05$ was significant. LMP1 scoring and intensity were non-normally distributed (Shapiro-Wilk $W = 0.589$ and 0.581, respectively, both $P < 0.0001$), so non-parametric methods were used throughout. Fisher's exact test compared LMP1 prevalence between groups as an exact alternative valid regardless of expected cell counts; the Mann-Whitney U test compared ordinal scores between independent groups, with effect size reported as rank-biserial r; the Kruskal-Wallis H test with Bonferroni-corrected post-hoc comparisons compared three groups; and Spearman's rank correlation (ρ) assessed monotonic associations. LMP1–EBER paired-marker agreement was quantified by Cohen's κ (interpreted per Landis and Koch: <0.20 slight; 0.21–0.40 fair; 0.41–0.60 moderate; 0.61–0.80 substantial; >0.80 almost perfect), with McNemar's test for systematic asymmetry of discordance. Odds ratios with 95% confidence intervals were computed where feasible.

RESULTS

Cohort Characteristics

The two study groups were demographically comparable. The 50 OSCC cases comprised 32 men and 18 women, with a mean age of 56.2 ± 13.4 years (median 55), while the 50 controls comprised an identical 32 men and 18 women, with a mean age of 58.5 ± 15.4 years (median 56). Age did not differ between cases and controls (Mann-Whitney $U = 1129.0$, $P = 0.405$), and the sex distribution was identical (Fisher's exact, $P = 1.000$), removing age and sex as group-level confounders of the case-control comparison.

LMP1 Expression: OSCC versus Controls

Immunohistochemistry revealed positive LMP1 staining in 13 of 50 OSCC cases (26.0%) but in none of the 50 healthy controls (0.0%) (Table 1, Figure 1). The association was highly significant (Fisher's exact test, $P = 0.0001$). Because no control expressed LMP1, the point-estimate odds ratio was undefined; the Haldane–Anscombe-corrected OR was 36.36 (95% CI: 2.09–631.21), the wide interval reflecting the zero-count control cell. The Mann-Whitney U test confirmed higher LMP1 scores in OSCC than in controls ($U = 1575.0$, $P = 0.0001$; rank-biserial $r = 0.26$). The 26% absolute difference identifies LMP1 as a marker of malignant rather than benign oral tissue in this cohort.

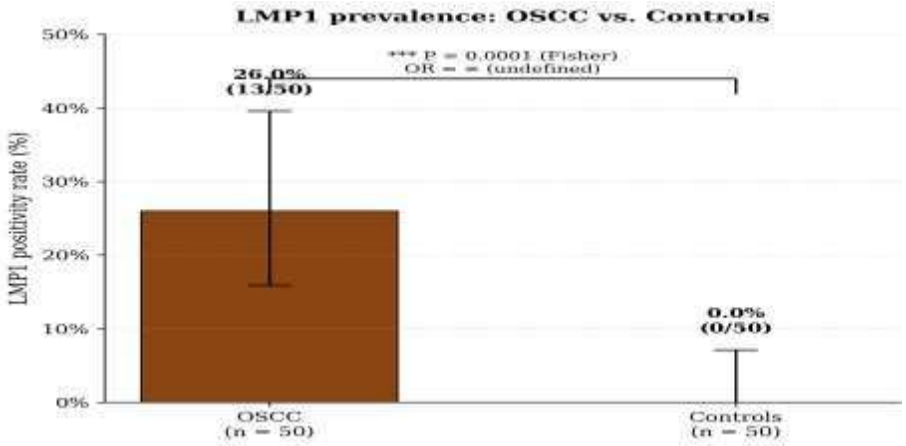

Figure 1. LMP1 positivity rate in OSCC cases (26.0%) versus normal controls (0.0%). The difference was highly significant (Fisher's exact test, P = 0.0001). Error bars represent 95% Wilson confidence intervals for proportions.

LMP1 Scoring and Intensity Distribution

Among the 13 LMP1-positive cases, the scoring distribution was score 1 (1–25%) in 5 cases, score 2 (26–50%) in 6, and score 3 (>50%) in 2; intensity was weak in 2, moderate in 5, and strong in 6 (Table 2). The mean LMP1 score across all OSCC was 0.46 ± 0.86 versus 0.00 in controls, and the mean intensity 0.60 ± 1.09 versus 0.00. Both differed significantly between groups (Mann-Whitney U = 1575.0, P = 0.0001). Moderate-to-strong expression (score ≥2) was present in 8 of 13 positive cases (61.5%), indicating that when EBV is oncogenically active it frequently produces substantial oncoprotein. Across all 50 cases, scoring and intensity were strongly correlated (Spearman ρ = 0.987, P < 0.0001); this coefficient is, however, inflated by the 37 double-negative cases, and within the 13 positive cases the correlation was moderate and non-significant (ρ = 0.468, P = 0.107).

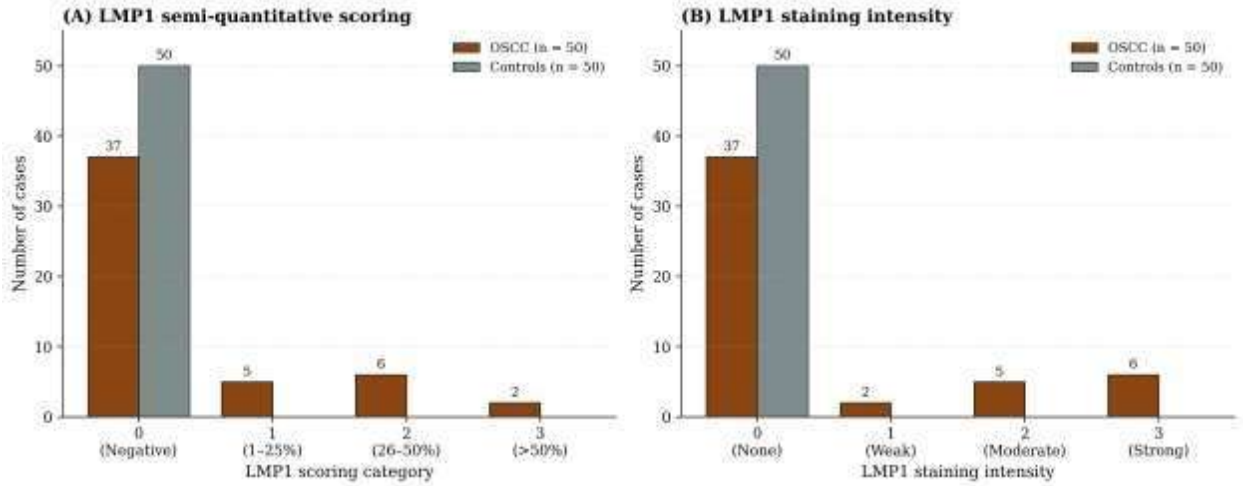

Figure 2. Distribution of LMP1 semi-quantitative scoring (A) and staining intensity (B) in the OSCC and control groups. All positive expression was confined exclusively to the OSCC group.

LMP1 Expression by Clinicopathological Parameters

LMP1 positivity was highest in Grade 2 (moderately differentiated) tumours (5/9, 55.6%), compared with Grade 1 (7/33, 21.2%) and Grade 3 (1/8, 12.5%) (Table 3; Figures 3–4); mean scores were 0.39 ± 0.83, 1.00 ± 1.12, and 0.13 ± 0.35, respectively. The Kruskal-Wallis test was not significant (H = 5.230, df = 2, P = 0.073), and Dunn's post-hoc test with Bonferroni correction showed no significant pairwise difference (Grade 1 versus 2, P = 0.129; Grade 2 versus 3, P = 0.114; Grade 1 versus 3, P = 1.000). Spearman's correlation between grade and LMP1 score was also non-significant (ρ = 0.073, P = 0.613). The apparently higher rate at Grade 2 therefore represents a non-significant trend rather than an established gradient, an interpretation further constrained by the small Grade 2 (n = 9) and Grade 3 (n = 8) subgroups.

No difference in LMP1 expression was observed between males (9/32, 28.1%) and females (4/18, 22.2%) (Fisher's exact P = 0.746, OR = 1.37; Mann-Whitney U = 310, P = 0.572; rank-biserial r = 0.08, negligible) (Figure 5). LMP1 score did not correlate with age (Spearman ρ = 0.149, P = 0.303), and LMP1-positive cases were of similar age to negative cases (59.6 ± 15.9 vs 55.0 ± 12.4 years; Mann-Whitney U = 293.0, P = 0.249) (Figure 6).

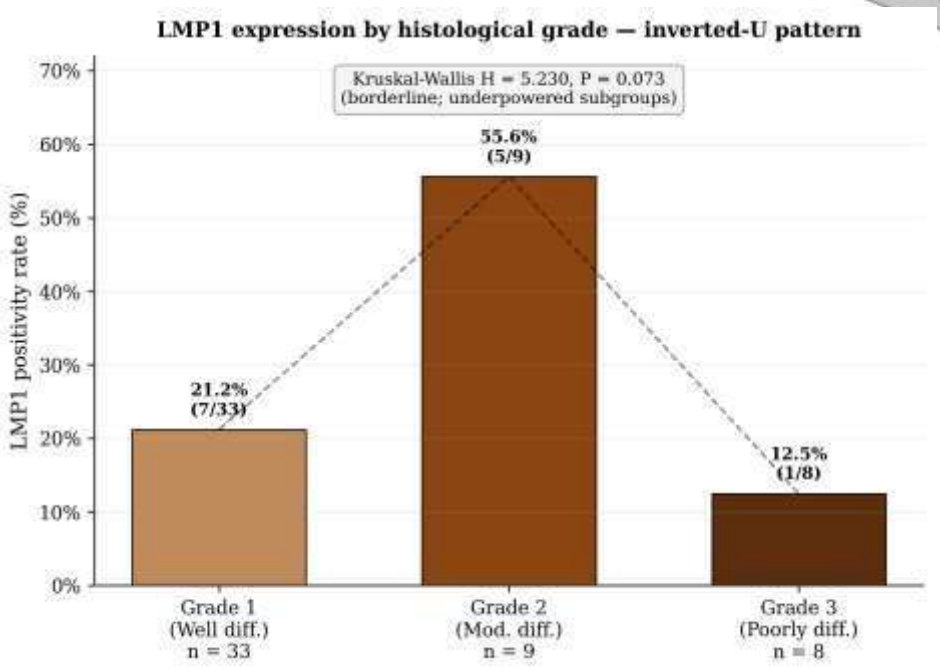

Figure 3. LMP1 positivity by histological grade. Positivity was numerically highest at Grade 2 (moderately differentiated, 55.6%) versus Grade 1 (21.2%) and Grade 3 (12.5%), but the difference was not statistically significant (Kruskal-Wallis H = 5.230, P = 0.073; Spearman ρ = 0.073, P = 0.613) and the Grade 2 estimate rests on nine patients.

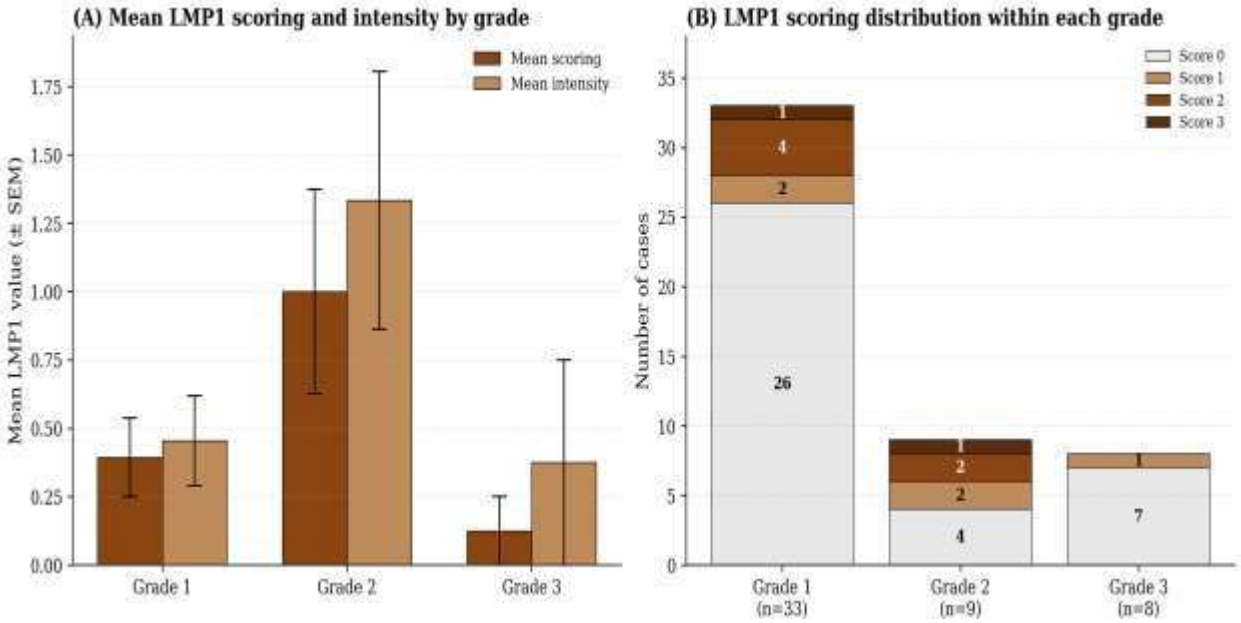

Figure 4. Detailed LMP1 expression by histological grade. (A) Mean LMP1 scoring and staining intensity (± SEM) across grades, with peak expression in Grade 2. (B) Proportional distribution of LMP1 scoring categories within each grade, showing concentration of higher scores (2–3) in moderately differentiated tumours.

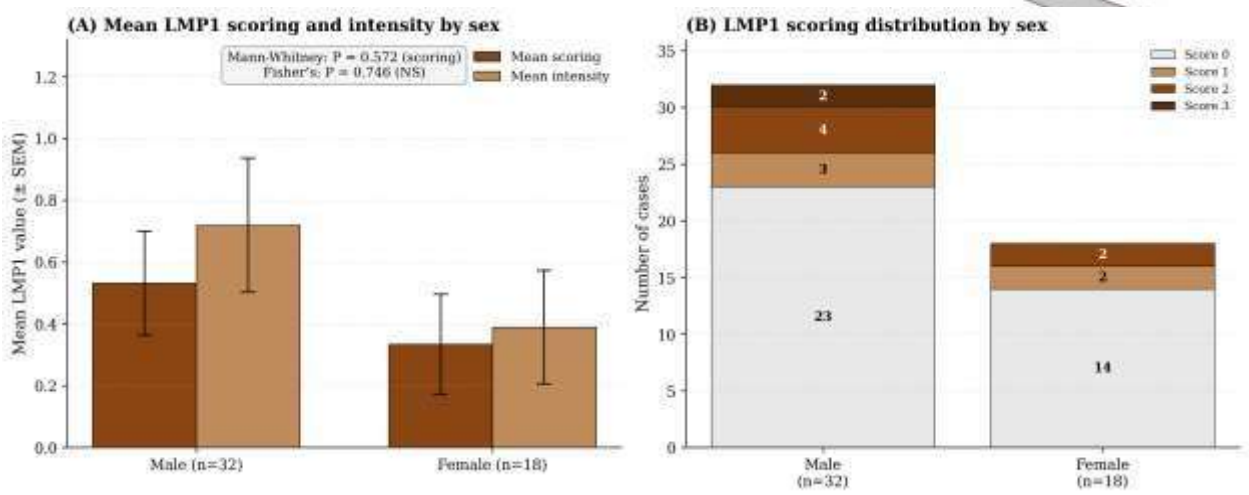

Figure 5. Detailed LMP1 expression by sex. (A) Mean LMP1 scoring and staining intensity (± SEM) in males versus females, with no significant difference (Mann-Whitney P = 0.572). (B) Proportional distribution of LMP1 scoring categories by sex.

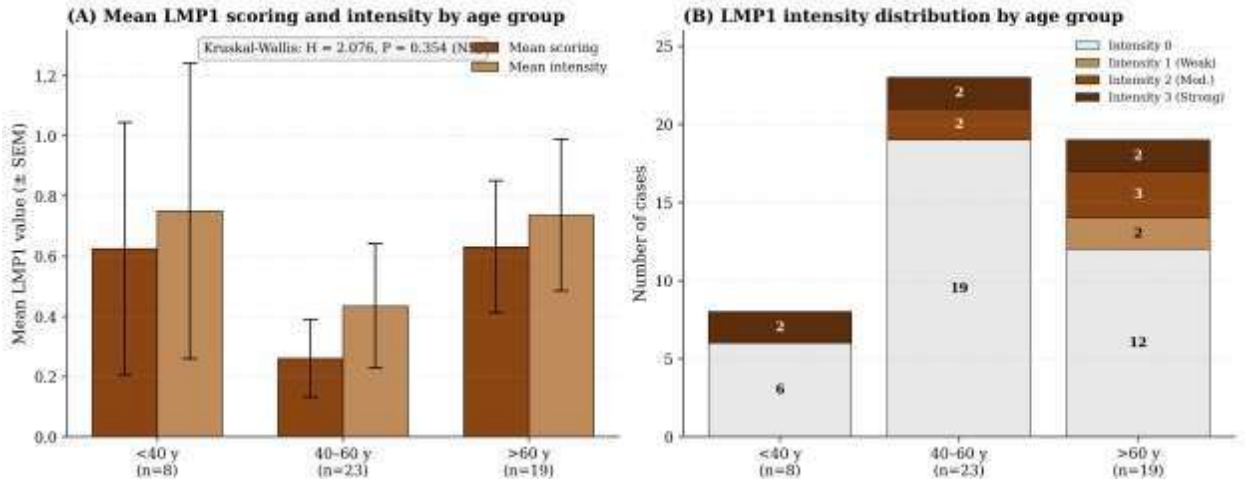

Figure 6. Detailed LMP1 expression by age group. (A) Mean LMP1 scoring and staining intensity (± SEM) across age groups, with a non-significant trend toward higher expression in patients older than 60 years. (B) Proportional distribution of LMP1 intensity categories by age group.

LMP1–EBER Concordance: Hierarchical (Nested) Architecture

Cross-tabulation of EBER and LMP1 status among the 50 OSCC cases revealed a strictly hierarchical relationship: all 13 LMP1-positive cases were simultaneously EBER-positive (13/13 on the LMP1-positive axis), whereas 9 of 22 EBER-positive cases (40.9%) lacked detectable LMP1, and no case showed the reverse pattern (LMP1+/EBER−) (Table 4, Figure 7). Observed agreement was 82% (41/50), with Cohen's κ = 0.618 (substantial agreement). McNemar's test confirmed systematic asymmetric discordance (P = 0.004; 9 EBER+/LMP1− versus 0 EBER−/LMP1+), and Fisher's exact test indicated strong non-random co-occurrence (P < 0.0001).

This one-directional discordance reflects the nested architecture of EBV latency: EBERs are transcribed in all four programmes and mark any latent infection, whereas LMP1 is restricted to Latency II and III [25]. LMP1 positivity therefore implies EBER positivity, but not the reverse. The 13 LMP1+/EBER+ cases (Stratum A; 26% of OSCC) are consistent with an active Latency II programme; the 9 LMP1−/EBER+ cases (Stratum B; 18%) with Latency 0 or I; and the remaining 28 cases (Stratum C; 56%) showed no EBV latency.

One caveat is required. EBER-ISH targets two non-coding RNAs present at roughly 10^4–10^6 copies per infected cell, whereas LMP1-IHC detects a single low-abundance protein. Some or all Stratum B specimens may therefore harbour

sub-threshold LMP1 below the detection limit of the present assay rather than a genuinely distinct latency programme. Discriminating a true latency difference from a detection-threshold effect cannot be resolved by the cross-sectional design and would require quantitative LMP1 mRNA in situ hybridisation, single-cell transcriptomics, or digital spatial profiling.

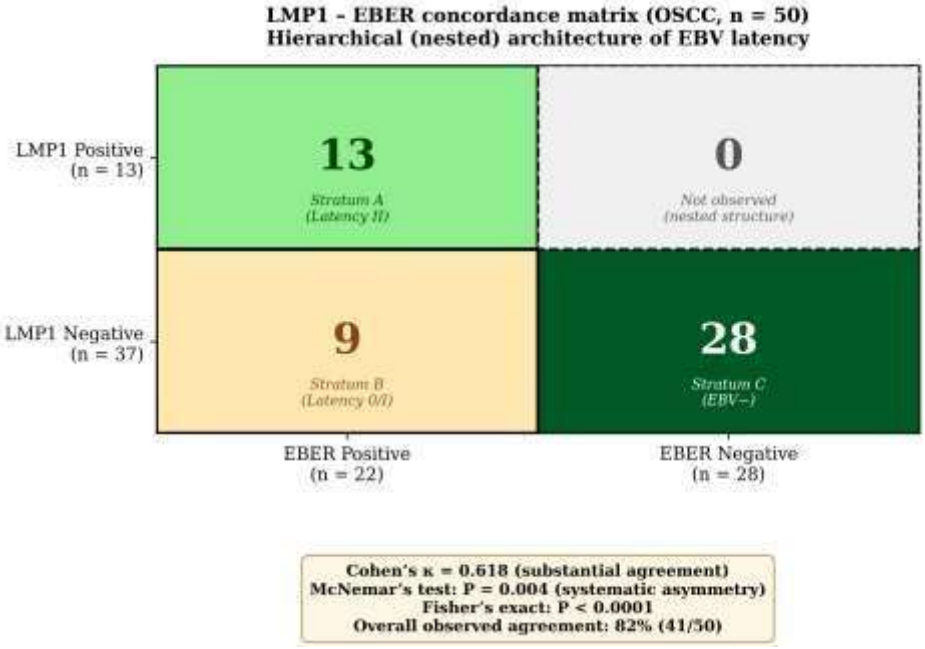

Figure 7. LMP1–EBER concordance matrix in 50 OSCC cases, illustrating the hierarchical (nested) architecture of EBV latency. All 13 LMP1-positive cases were EBER-positive (Stratum A, Latency II-consistent), while 9 EBER-positive cases lacked LMP1 (Stratum B, Latency 0/I-consistent). No LMP1+/EBER− case was observed, confirming the nested structure. Cohen's κ = 0.618; McNemar's P = 0.004.

EBV Marker Expression Profile

The expression profile across all 50 cases (Figure 8) delineated a coordinated EBER- and LMP1-co-expressing subgroup (Stratum A), an EBER-only subgroup (Stratum B), and an EBV-negative majority (Stratum C), corroborating the concordance analysis. The Spearman correlation matrix (Figure 9) was dominated by the all-case LMP1 score–intensity coefficient (ρ = 0.987), which is inflated by the double-negative cases (within-positive ρ = 0.468), with weak-to-negligible correlations with age (ρ = 0.149), sex, and grade.

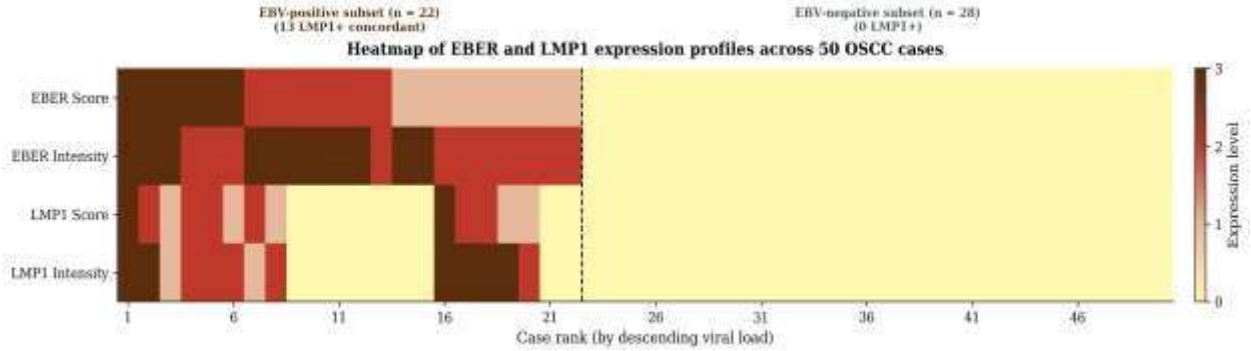

Figure 8. Heatmap of EBER and LMP1 expression profiles (scoring and intensity) across all 50 OSCC cases ranked by descending viral load. Colour intensity represents the expression level on a 0–3 scale. The three strata are visually distinguishable: Stratum A (EBER+/LMP1+, n = 13), Stratum B (EBER+/LMP1−, n = 9), and Stratum C (EBV−, n = 28).

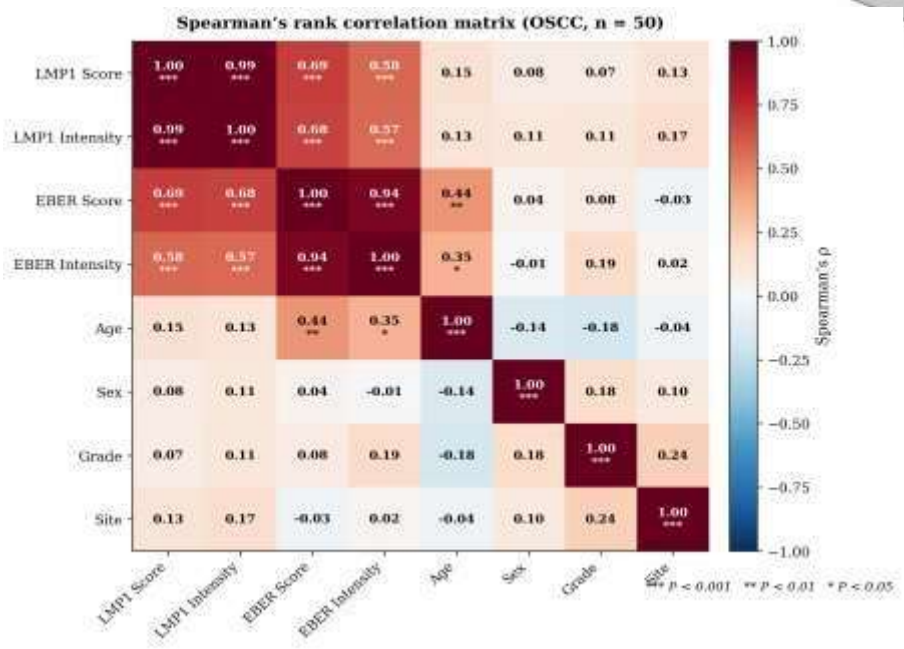

Figure 9. Spearman's rank correlation matrix for LMP1 expression and clinicopathological parameters in OSCC cases (n = 50). The all-case LMP1 score–intensity coefficient (ρ = 0.987) was the dominant feature of the matrix but is inflated by the 37 double-negative cases (within-positive ρ = 0.468); correlations with demographic and histological parameters were negligible.

Representative LMP1 Immunohistochemical Photomicrographs

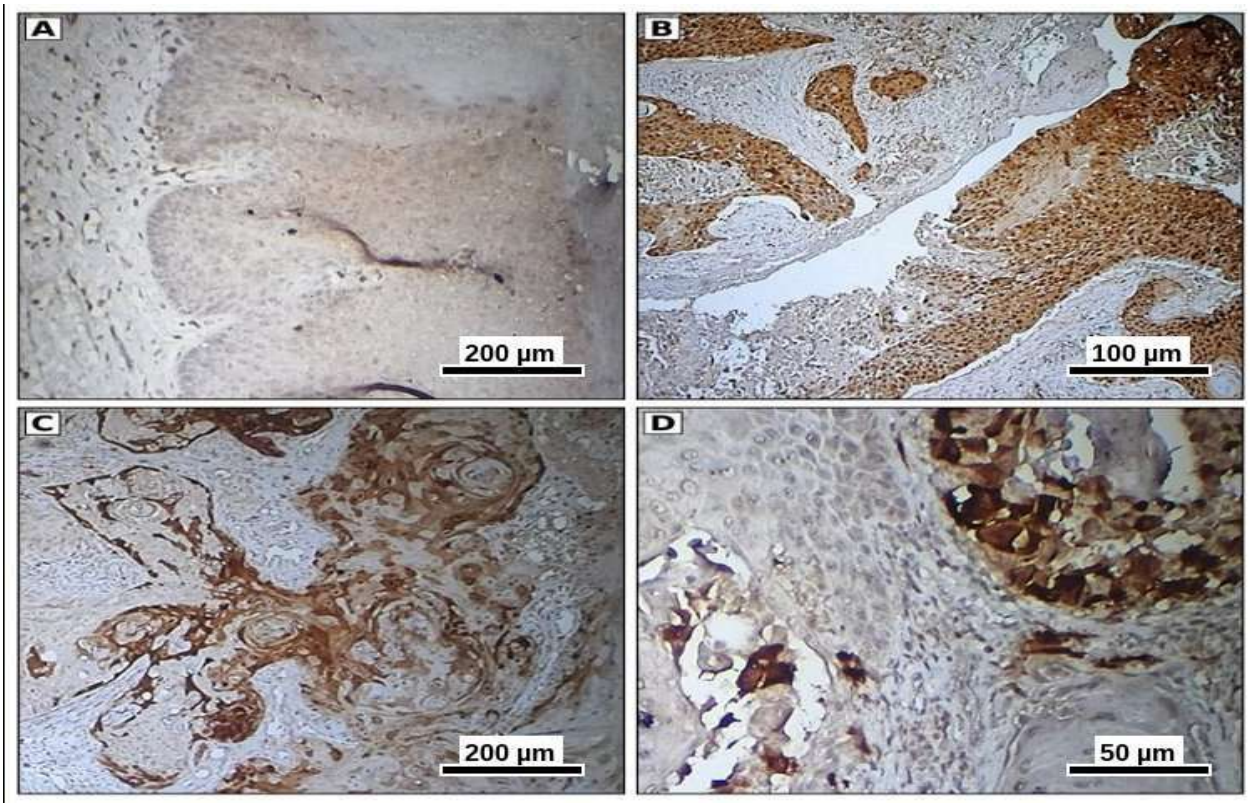

Figure 10. Representative immunohistochemical photomicrographs of LMP1 expression in OSCC and normal oral mucosa (DAB chromogen, Mayer's haematoxylin counterstain). (A) Negative control: normal oral mucosa with intact squamous

stratification and no DAB deposition in epithelium or stroma, indicating absent LMP1 expression (×200). (B) Moderately differentiated OSCC (Grade 2) with strong (3+) LMP1 expression: diffuse, intense cytoplasmic and membranous staining in more than 50% of neoplastic cells, predominantly membranous with cytoplasmic reinforcement, including at the tumour–stroma interface (×100). (C) Moderate (2+) LMP1 expression in well-differentiated OSCC (Grade 1): immunoreactivity at the periphery of keratin pearls with negative keratinised cores, a heterogeneous pattern (×100). (D) High-power view of granular cytoplasmic and membranous LMP1 staining in tumour-cell nests at the invasive front; intensely positive neoplastic cells are sharply demarcated from adjacent LMP1-negative stromal and inflammatory cells, confirming epithelial localisation and excluding lymphocyte contamination (×400).

TABLES

Table 1. LMP1 expression in OSCC and control groups.

Group	LMP1+ n (%)	LMP1− n (%)	Total	OR (95% CI)
OSCC (cases)	13 (26.0)	37 (74.0)	50	36.36 (2.09–631.21)†
Controls	0 (0.0)	50 (100)	50	—
Total	13 (13.0)	87 (87.0)	100	—

Fisher's exact test, P = 0.0001. Mann-Whitney U = 1575.0, P = 0.0001 (rank-biserial r = 0.26). †Point-estimate OR undefined (no control expressed LMP1); Haldane–Anscombe-corrected OR shown.

Table 2. LMP1 scoring and intensity distribution (OSCC vs controls).

Parameter	OSCC n (%)	Controls n (%)
Score 0 (0%)	37 (74.0)	50 (100)
Score 1 (1–25%)	5 (10.0)	0 (0.0)
Score 2 (26–50%)	6 (12.0)	0 (0.0)
Score 3 (>50%)	2 (4.0)	0 (0.0)
Mean score ± SD	0.46 ± 0.86	0.00 ± 0.00
Intensity weak (1+)	2 (4.0)	0 (0.0)
Intensity moderate (2+)	5 (10.0)	0 (0.0)
Intensity strong (3+)	6 (12.0)	0 (0.0)
Mean intensity ± SD	0.60 ± 1.09	0.00 ± 0.00

Mann-Whitney U = 1575.0, P = 0.0001 for both scoring and intensity. The all-case score–intensity coefficient (ρ = 0.987) is inflated by the 37 double-negative cases; within the 13 positive cases ρ = 0.468 (P = 0.107, not significant).

Table 3. LMP1 expression by clinicopathological parameters (OSCC cases, n = 50).

Parameter	Category	LMP1+ n/N (%)	Statistic	P
Grade	Well-diff. (I)	7/33 (21.2)	H = 5.230	0.073
	Mod.-diff. (II)	5/9 (55.6)		
	Poorly-diff. (III)	1/8 (12.5)	ρ = 0.073	0.613
Sex	Male	9/32 (28.1)	OR = 1.37	0.746
	Female	4/18 (22.2)	U = 310	0.572
Age (yr)	LMP1+ vs LMP1− (mean)	59.6 vs 55.0	U = 293.0	0.249

Non-parametric tests throughout. Grade rates compared by Kruskal-Wallis (H = 5.230, P = 0.073); Dunn's post-hoc with Bonferroni correction was non-significant (Grade 1 vs 2, P = 0.129; Grade 2 vs 3, P = 0.114). Spearman ρ tests the monotonic grade trend (ρ = 0.073, P = 0.613) and continuous age (ρ = 0.149, P = 0.303). Sex compared by Fisher's exact and Mann-Whitney U. No comparison reached P < 0.05.

Table 4. LMP1–EBER concordance matrix (OSCC cases, n = 50).

	LMP1+	LMP1−	Total
EBER+	13 (Stratum A)	9 (Stratum B)	22
EBER−	0	28 (Stratum C)	28
Total	13	37	50

Observed agreement 41/50 = 82%. Cohen's κ = 0.618 (substantial). McNemar's test P = 0.004 (systematic asymmetric discordance). Fisher's exact test P < 0.0001. The nine EBER+/LMP1− cases (Stratum B) may represent a Latency 0/I programme or sub-threshold LMP1 below the IHC detection limit.

Table 5. Master summary of statistical analyses (n = 50 OSCC).

Comparison	Primary test	Statistic	P-value	Significance
LMP1: OSCC vs control	Fisher's exact	P = 0.0001	0.0001*	Significant
LMP1 score (OSCC vs ctrl)	Mann-Whitney U	U = 1575.0	0.0001*	Significant
Grade	Kruskal-Wallis	H = 5.230	0.073	Not significant
Sex	Fisher / Mann-Whitney	OR = 1.37	0.746	Not significant
Age (continuous)	Spearman ρ	ρ = 0.149	0.303	Not significant
Score ↔ intensity	Spearman ρ	ρ = 0.987	<0.0001*	Significant
LMP1–EBER concordance	Cohen's κ / McNemar	κ = 0.618	0.004*	Significant

*Non-parametric tests were used as primary analyses throughout, given the confirmed non-normality of the LMP1 data (Shapiro-Wilk W = 0.589, P < 0.0001). Bonferroni correction was applied to post-hoc pairwise comparisons. *P < 0.05 (two-tailed).*

DISCUSSION

LMP1 immunohistochemical expression was detected in 26.0% of Iraqi OSCC cases, with complete absence in normal controls (Fisher's exact P = 0.0001). Scoring and intensity were strongly correlated across all cases (ρ = 0.987), although this coefficient is inflated by the predominance of double-negative cases (within-positive ρ = 0.468). Our scoring approach aligns with published staining intensity–distribution methods [26], and the long-standing controversy in the literature is partly attributable to methodological differences in EBV detection [12].

The absolute absence of LMP1 in normal mucosa supports its status as a marker of malignant transformation; LMP1 has previously been reported in 62.5% of epithelial dysplasias and 81.8% of OSCC [27]. Detection of EBV protein within tumour cells argues for a direct association rather than a background presence [13]. Although low EBV genome copy numbers have been used to argue for a passenger role [28], LMP1, as the major oncoprotein, is more plausibly a participant in carcinogenesis when overexpressed in a substantial OSCC subset [7].

The most informative finding is the strictly hierarchical LMP1–EBER concordance: all 13 LMP1-positive cases were EBER-positive, but 9 of 22 EBER-positive cases lacked LMP1 (κ = 0.618; McNemar P = 0.004). This faithfully reflects the nested architecture of EBV latency. The 13 LMP1+/EBER+ cases (Stratum A; 26%) are consistent with an active Latency II programme — EBNA1, LMP1, LMP2, BARTs, and EBERs — conventionally associated with nasopharyngeal carcinoma and Hodgkin lymphoma [23,25]. The 9 LMP1−/EBER+ cases (Stratum B; 18%) are most consistent with Latency 0 or I, and the remaining 28 cases (Stratum C; 56%) showed no EBV latency.

Several alternatives must be weighed before these assignments are regarded as definitive. Without EBNA2 or EBNA3 assessment, a Latency III profile cannot be formally excluded, although epithelial cancers predominantly express Latency II or a variant [23,25]. Because BRLF1 can induce LMP1 during lytic replication [25], an abortive-lytic contribution cannot be excluded without immediate-early antigen testing. Most consequentially, EBER-ISH and LMP1-IHC differ markedly in analytical sensitivity: EBER-ISH targets two non-coding RNAs present at roughly 10^4–10^6 copies per infected cell, whereas LMP1-IHC detects a single low-abundance protein. Some or all Stratum B specimens may therefore carry sub-threshold LMP1 rather than a genuinely distinct programme, and the cross-sectional design cannot resolve a true latency difference from a detection threshold; doing so would require quantitative LMP1 mRNA in situ hybridisation, single-cell transcriptomics, or digital spatial profiling. Requiring epithelial LMP1 localisation addresses the passenger-virus and bystander-lymphocyte concerns [13,7], and the retention of LMP1 in 26% of cases argues against complete viral loss in this subset, even if Stratum B may represent intermediate hit-and-run stages [16].

EBV oncogenesis appeared sex-independent, consistent with the prevailing view that its carcinogenic effect is similar in both sexes [28]. A non-significant trend toward higher LMP1 positivity above 60 years accords with reports of slightly increased risk in older subjects [28] and with immunosenescence-related vulnerability to viral reactivation [29], but the present sample could not resolve the matter.

The non-significant trend toward peak LMP1 expression at Grade 2 is biologically interpretable but must be read cautiously: the omnibus test did not reach significance (P = 0.073) and the Grade 2 estimate rests on nine patients, while small studies tend to overestimate effect sizes [30], so larger cohorts are needed [7]. LMP1 is essential for transformation yet dispensable once cells are transformed [18], is concentrated in the basal layer of premalignant lesions [7,27], and is self-limiting at high levels because abundant LMP1 induces cytostasis, autophagy, and apoptosis in epithelial cells [20]. This double-edged behaviour can account for a peak at intermediate differentiation and suppression in poorly differentiated tumours. As genomic instability — to which LMP1 contributes through Egr-1-mediated upregulation of AID [20] — secures tumour autonomy, the virus may be downregulated or lost, while stable epigenetic reprogramming persists [16]; whether LMP1-driven DNMT activation leaves a heritable methylation imprint after LMP1 silencing is addressed in a companion investigation of sFRP promoter methylation in this cohort (in preparation).

Several limitations apply. The sample (n = 50 per group) limits statistical power, and the borderline grade association (P = 0.073) likely reflects this rather than a true null. The single-centre retrospective design from Najaf constrains generalisability to other Iraqi or Middle Eastern populations. Definitive latency typing requires additional markers (EBNA2, EBNA3, LMP2A); only LMP1 and EBER were assessed. The detection-threshold explanation for Stratum B cannot be formally excluded without quantitative LMP1 measurement. HPV co-infection status was not evaluated, and dual-staining co-localisation or laser-capture microdissection was not performed, although morphological assessment strongly supported epithelial localisation. The cross-sectional case-control design precludes causal inference; prospective cohorts with longitudinal follow-up are required to establish temporality.

CONCLUSION

EBV demonstrates predominant epithelial localisation and active oncogenic signalling in a defined subset of Iraqi OSCC, establishing the virus as a prominent regional risk determinant. The hierarchical LMP1–EBER concordance (Cohen's κ = 0.618; McNemar P = 0.004) identifies three biologically distinct strata: Stratum A (LMP1+/EBER+, 26%), consistent with an active Latency II programme; Stratum B (LMP1−/EBER+, 18%), consistent with Latency 0 or I (with sub-threshold LMP1 a plausible alternative); and Stratum C (EBV−, 56%). These findings justify multicentre studies with expanded viral marker panels (EBNA1, EBNA2, LMP2A, BART transcripts) to refine latency assignment and to investigate the downstream epigenetic consequences of EBV infection in Iraqi oral carcinogenesis.

Ethical Approval Declaration

The study protocol was approved by the Research Administration Section of the Najaf Health Directorate (approval No. 19688, dated 17 September 2024) and was conducted in accordance with the Declaration of Helsinki (1964) and its later amendments.

Assignment

As the study was retrospective and used archival FFPE blocks with no patient contact, the requirement for individual informed consent was waived by the ethics committee. All specimens were anonymised and handled confidentially, and the information was used solely for the purposes of this study.

Statements and Declarations

Conflict of Interest: The authors declare no conflict of interest.

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Author Contributions: A.R.A. conceived and designed the study, performed the experiments, analysed the data, and drafted the manuscript. M.N.M. supervised the project and critically revised the manuscript. R.J.A. contributed to the histopathological and immunohistochemical evaluation. All authors read and approved the final manuscript.

Data Availability: All data generated or analysed during this study are included in this article. Additional anonymised data are available from the corresponding author upon reasonable request, subject to the institutional data-sharing policies of Al-Sadder Medical City, Najaf, Iraq.

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