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Apoptosis-Associated Changes and Biopsy-Tissue Microarray Correlations in HPV-Associated Cervical Lesions among Sudanese Women

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Abstract:

Background: *Apoptosis-associated changes and tissue microarray (TMA) performance remain insufficiently characterized in HPV-associated cervical lesions from resource-limited settings. This study evaluated morphologic apoptosis, TUNEL scores, and Bcl-2 expression across lesion severity and compared TMA measurements with conventional biopsy sections.*

Methods: *This cross-sectional diagnostic-pathology study included 200 HPV-positive Sudanese women. Consensus histology classified specimens as negative/benign (n = 20), LSIL/CIN1 (n = 30), HSIL/CIN2-3 (n = 100), or invasive squamous cell carcinoma (SCC; n = 50). Morphologic apoptosis and TUNEL were scored on 0-3 ordinal scales, and Bcl-2 was quantified as percentage expression. Sixty cases had paired biopsy and TMA measurements. Kruskal-Wallis tests, Spearman correlations, and an exploratory ridge logistic regression model were used.*

Results: *Morphologic apoptosis and TUNEL were higher in high-grade and invasive lesions than in low-grade or benign tissue and correlated with ordered severity ($r = 0.426$ and $r = 0.375$, respectively; both $p < 0.001$). Bcl-2 expression was nonlinear: the mean increased from 20.00% in negative/benign tissue to 59.89% in HSIL/CIN2-3, then declined to 30.78% in invasive SCC. In the exploratory invasive-SCC model, p53 and Ki-67 had the largest positive standardized odds ratios, whereas Bcl-2 was inversely associated. Biopsy-TMA rank correlations ranged from 0.569 to 0.728 for immunohistochemical markers; TUNEL yielded $r = 1.000$.*

Conclusions: *Advanced lesions showed a high-turnover phenotype rather than evidence of effective regression. Bcl-2 identified a transition between preinvasive and invasive disease. TMA correlations support tissue-efficient research use; however, correlation alone does not establish agreement or diagnostic interchangeability with whole sections.*

Keywords: apoptosis; Bcl-2; TUNEL; tissue microarray; cervical intraepithelial neoplasia; cervical cancer; human papillomavirus; Sudan

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1. INTRODUCTION

Persistent high-risk human papillomavirus (HPV) infection is the principal initiating event in most cervical squamous carcinomas, but viral persistence alone does not explain progression from productive infection to high-grade squamous intraepithelial lesion (HSIL) and invasive carcinoma [1,2]. Deregulated E6 and E7 expression disturbs p53 and retinoblastoma-pathway control, expands proliferating cell populations and alters epithelial maturation [3,4]. Invasion, however, also involves cell death, clonal selection, immune pressure, hypoxia and interaction with the stromal microenvironment.

Apoptosis is a regulated form of cell death essential to tissue homeostasis. Its morphological features include cell shrinkage, chromatin condensation, karyorrhexis and formation of apoptotic bodies [5]. Terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) detects DNA fragmentation in situ and is widely used as an apoptosis-associated tissue assay [6]. Neither method is completely specific in isolation. TUNEL can label non-apoptotic DNA damage, and morphological assessment is influenced by sampling, tissue preservation and observer interpretation.

In cancer, increased apoptosis does not necessarily indicate tumor regression. Rapidly proliferating tumors can show high rates of both cell production and cell loss; treatment-independent apoptosis may reflect genomic damage, hypoxia, immune attack or selection of resistant clones. Apoptotic signaling can therefore coexist with, and in some settings contribute to, tumor progression [7,8]. Cervical lesions should consequently be interpreted as dynamic tissue systems in which proliferation and cell death are simultaneously deregulated.

Bcl-2 provides a complementary view of this biology. It is an anti-apoptotic mitochondrial protein that promotes cell survival, but its expression need not increase monotonically with tumor severity. High-grade intraepithelial lesions may depend on Bcl-2-mediated survival while remaining confined to the epithelium; invasive clones may subsequently acquire alternative survival mechanisms, increased heterogeneity or different interactions with stroma and hypoxia. Previous studies of cervical lesions have reported complex, pathway-specific patterns rather than uniform progression of all apoptosis-related markers [9-11].

Tissue microarray (TMA) technology is attractive for biomarker research in resource-limited pathology services. Multiple small cores can be stained in the same run, reducing reagent use, preserving donor blocks and limiting batch-to-batch variation [12,13]. These advantages are particularly relevant where antibodies and detection reagents are expensive or intermittently available. Yet cervical lesions are spatially heterogeneous, and small cores may miss focal invasion, lesion edges, glandular extension, necrosis or patchy staining. TMA performance must therefore be compared with conventional sections before it is used as a research platform, and statistical correlation must not be confused with agreement.

This study had two objectives. First, it examined morphological apoptosis, TUNEL score and Bcl-2 expression across four histological categories of HPV-associated cervical disease. Second, it assessed rank correlation between paired TMA and conventional biopsy-section measurements. We hypothesized that apoptosis-associated indices would be higher in high-grade and invasive lesions, that Bcl-2 would show a nonlinear pattern, and that TMA measurements would correlate moderately with whole-section results while remaining unsuitable as a replacement for diagnostic morphology.

2. MATERIALS AND METHODS

2.1 Study design and participants

This cross-sectional diagnostic-pathology analysis included a referral cohort of 200 HPV-positive Sudanese women. Recruitment comprised 80 participants (40.0%) from Soba University Hospital and 120 (60.0%) from decentralized referral pathways encompassing River Nile State (including Shendi/River Nile RICC), Northern State, and cities in Eastern Sudan. The analysis evaluated biologic gradients within a diagnostic-referral population; lesion proportions were not interpreted as community prevalence.

Women were eligible when high-risk HPV positivity was documented, cervical cytology and/or colposcopic assessment was available, a cervical biopsy or excision provided reliable histology, and sufficient FFPE material remained for the relevant biomarker analysis. Specimens with inadequate fixation, insufficient lesional tissue, prior treatment, a non-cervical primary tumor or no defensible final diagnosis were excluded.

2.2 Histopathology and apoptosis assessment

Hematoxylin and eosin-stained sections were reviewed by two pathologists when dual review was available, and discordant diagnoses were resolved by consensus. Final categories were negative/benign, LSIL/CIN1, HSIL/CIN2-3, and invasive SCC. The invasive-SCC endpoint compared invasive carcinoma with all noninvasive categories.

Morphologic apoptosis was scored on an ordinal scale from 0 to 3 based on apoptotic bodies, chromatin condensation, karyorrhexis, and cellular fragmentation in preserved lesional areas. TUNEL staining was performed on formalin-fixed, paraffin-embedded sections to identify in situ DNA fragmentation and was also recorded on a 0-3 ordinal scale. Necrotic, cauterized, folded, or poorly preserved areas were excluded to reduce nonspecific interpretation.

Bcl-2 immunohistochemistry was evaluated as the percentage of lesional cells showing cytoplasmic expression. Positive and negative controls were included in staining runs. p53 and Ki-67 percentages were retained as covariates in the exploratory invasive-SCC model because they represented tumor-suppressor-pathway disturbance and proliferation, respectively; their primary diagnostic analysis is reported separately.

2.3 Tissue microarray assessment

Representative donor blocks were selected after review of the corresponding H&E sections. Lesional areas were marked, and cores were taken from regions judged to contain the relevant epithelial abnormality while avoiding necrosis and technically compromised tissue. TMA sections were processed using the same marker-specific staining framework where feasible. Lost cores, absent epithelium and uninterpretable staining were treated as technical missingness rather than negative expression.

Paired conventional biopsy-section and TMA measurements were available for 60 cases. The paired variables were p14, p15, p16, p53, Ki-67, Bcl-2 and TUNEL. TMA was evaluated as a research-economy platform; whole-section morphology remained the diagnostic standard for architecture, invasion and lesion heterogeneity.

2.4 Statistical analysis

Continuous and ordinal variables were summarized using means and standard deviations to retain comparability with the study dataset. Kruskal-Wallis tests assessed

differences across the four histologic groups, and Spearman rank correlation quantified associations with ordered severity. Spearman correlation was also used for paired biopsy-TMA measurements because several variables were ordinal or nonnormally distributed. An exploratory L2-regularized logistic regression model was fitted for invasive SCC. Age, p53, Ki-67, Bcl-2, and TUNEL were standardized to a mean of zero and a standard deviation of one before modeling. Odds ratios therefore represent the change in odds per one-standard-deviation increase in the predictor. The model AUC was calculated in the derivation dataset and is reported as apparent discrimination; no independent or cross-validated estimate was available. Two-sided p values < 0.05 were considered statistically significant.

Correlation was interpreted as association, not agreement. Demonstration of interchangeability would require complementary analyses such as intraclass correlation, paired bias estimation or Bland-Altman limits, together with marker-specific acceptability criteria [14].

3. RESULTS

3.1 Apoptosis-associated findings across lesion groups

The four histologic groups comprised negative/benign (n = 20), LSIL/CIN1 (n = 30), HSIL/CIN2-3 (n = 100), and invasive SCC (n = 50). Morphologic apoptosis and TUNEL scores were substantially higher in HSIL and invasive SCC than in negative/benign and LSIL/CIN1 specimens (Table 1; Figure 1). Morphologic apoptosis correlated with ordered severity at r = 0.426, and TUNEL at r = 0.375; both p < 0.001. Neither measure increased further between HSIL and invasive SCC: mean morphologic apoptosis was identical in the two groups, and mean TUNEL was slightly lower in invasive SCC than in HSIL.

Table 1. Apoptosis-associated measurements across final histology groups.

Variable	Negative/benign (n = 20)	LSIL/CIN1 (n = 30)	HSIL/CIN2-3 (n = 100)	Invasive SCC (n = 50)	Spearman r	p value
Morphological apoptosis (0-3)	0.65 ± 0.49	0.93 ± 0.87	1.98 ± 0.85	1.98 ± 0.80	0.426	<0.001
TUNEL score (0-3)	0.90 ± 0.64	1.10 ± 0.55	1.98 ± 0.80	1.90 ± 0.84	0.375	<0.001
Bcl-2 expression (%)	20.00 ± 8.89	46.60 ± 13.54	59.89 ± 14.26	30.78 ± 20.10	-0.035	0.624

Values are mean ± SD. Spearman r represents correlation with the ordered four-level histological severity scale.

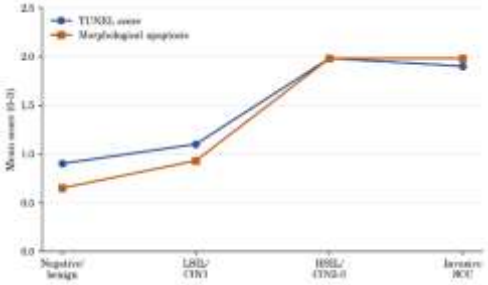

Figure 1. *Morphological apoptosis and TUNEL scores across final histology groups.*

The results indicate a high-turnover phenotype in advanced lesions rather than a simple protective response. Increased evidence of DNA fragmentation and apoptotic morphology coexisted with the marked proliferative activity documented in the same HSIL and invasive specimens.

3.2 Nonlinear Bcl-2 expression

Bcl-2 differed significantly among histologic groups by Kruskal-Wallis testing ($p < 0.001$), but it did not correlate monotonically with ordered severity ($r = -0.035$, $p = 0.624$). Mean expression increased from 20.00% in negative/benign tissue to 46.60% in LSIL/CIN1 and 59.89% in HSIL/CIN2-3, then decreased to 30.78% in invasive SCC (Figure 2). The larger standard deviation in invasive SCC also suggested greater heterogeneity.

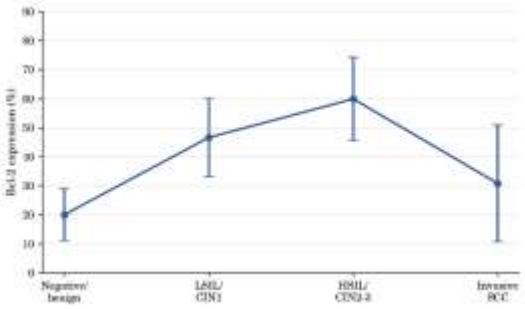

Figure 2. *Nonlinear Bcl-2 expression across lesion groups; points represent means and error bars represent one standard deviation.*

The divergence between Bcl-2 and the two apoptosis indices was biologically meaningful. TUNEL and morphological apoptosis were higher in advanced disease, whereas Bcl-2 was most strongly expressed in the pre-invasive HSIL category. The markers therefore captured different components of tissue turnover and should not be interpreted interchangeably.

3.3 Exploratory model for invasive SCC

In the regularized model, p53 and Ki-67 had the largest positive standardized odds ratios for invasive SCC (5.406 and 5.426, respectively). Older age was also positively associated (OR = 2.228 per SD). Bcl-2 showed an inverse association (OR = 0.196), whereas TUNEL was approximately neutral after adjustment (OR = 0.853). The apparent model AUC was 0.978 (Table 2; Figure 3).

Table 2. Exploratory standardized ridge logistic regression model for invasive SCC.

Predictor (per +1 SD)	Standardized OR	Coefficient	Interpretation
Age (years)	2.228	0.801	Positive association
p53 (%)	5.406	1.687	Positive association
Ki-67 (%)	5.426	1.691	Positive association
Bcl-2 (%)	0.196	-1.627	Inverse association

Predictor (per +1 SD)	Standardized OR	Coefficient	Interpretation
TUNEL score	0.853	-0.159	Approximately neutral after adjustment
Apparent model AUC	0.978	-	Derivation-sample discrimination

Predictors were standardized before modeling. Penalized coefficients are descriptive; conventional confidence intervals and p values were not estimated.

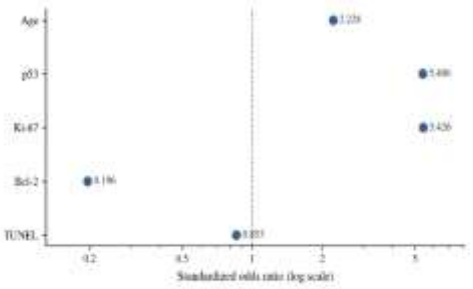

Figure 3. *Standardized odds ratio point estimates from the exploratory ridge logistic regression model for invasive SCC. Confidence intervals were not available.*

3.4 Biopsy-TMA rank correlations

Among the 60 paired cases, all biopsy-TMA rank correlations were statistically significant (Table 3; Figure 4). For immunohistochemical markers, correlations ranged from r = 0.569 for Bcl-2 to r = 0.728 for Ki-67. p15, p16, and p53 showed correlations of 0.693, 0.673, and 0.677, respectively. TUNEL yielded r = 1.000; this value should be interpreted cautiously because both platforms used the same coarse 0-3 ordinal scale, which can preserve rank order without demonstrating close numerical agreement.

Table 3. Spearman correlations between conventional biopsy-section and TMA measurements.

Biopsy marker	Paired TMA measure	Complete pairs	Spearman r	p value
p14 (%)	TMA p14 mean	60	0.587	<0.001
p15 (%)	TMA p15 mean	60	0.693	<0.001
p16 (%)	TMA p16 mean	60	0.673	<0.001
p53 (%)	TMA p53 mean	60	0.677	<0.001
Ki-67 (%)	TMA Ki-67 mean	60	0.728	<0.001
Bcl-2 (%)	TMA Bcl-2 mean	60	0.569	<0.001
TUNEL score	TMA TUNEL mean	60	1.000	<0.001

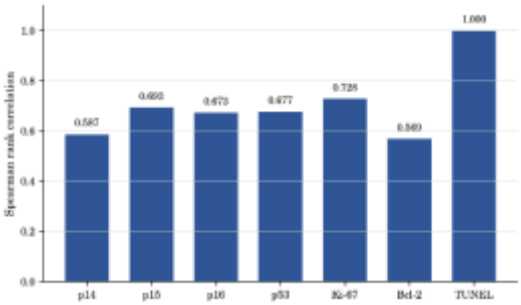

Figure 4. *Biopsy-TMA Spearman rank correlations in 60 paired cases.*

These results support common rank ordering of many cases across the two platforms, particularly for Ki-67, p15, p53 and p16. They do not show that TMA and whole sections generate equivalent absolute values, nor that a diagnostic threshold can be transferred unchanged between platforms.

4. DISCUSSION

This study provides two complementary observations. First, advanced HPV-associated cervical lesions showed increased apoptosis-associated morphology and TUNEL labeling alongside strong proliferative activity. Second, TMA measurements were moderately correlated with conventional biopsy-section results for most markers. Both observations have potential value for resource-limited biomarker research, but neither justifies replacing morphology-based diagnosis or whole-section assessment.

The apoptosis findings are consistent with high tumor-cell turnover. Morphological apoptosis and TUNEL were higher in HSIL and invasive SCC than in benign and low-grade lesions, but they did not rise further from HSIL to invasion. This plateau is important. It indicates that apoptosis-associated signals identify advanced neoplastic biology but do not independently mark the transition through the basement membrane. In a rapidly proliferating lesion, substantial cell loss may coexist with net tissue expansion because production exceeds loss. Apoptosis can also contribute to clonal selection and remodeling of the tumor microenvironment [7,8].

TUNEL must be interpreted within its technical limitations. It detects DNA strand breaks rather than apoptosis as a single exclusive process. Necrosis, severe DNA damage, tissue handling and some repair processes can generate labeling. The use of matched morphology, exclusion of necrotic or cauterized areas and control sections improves interpretation but does not make TUNEL a stand-alone diagnostic test. The present results therefore support TUNEL as a research measure of altered tissue turnover, not as a clinical triage marker.

The most distinctive finding was the nonlinear Bcl-2 trajectory. Expression peaked in HSIL/CIN2-3 and declined after invasion. This pattern suggests that Bcl-2-mediated survival may be particularly relevant while high-grade clones persist within the epithelium. Invasive carcinomas may rely on alternative anti-apoptotic pathways, may contain more heterogeneous clones, or may experience microenvironmental

pressures that alter Bcl-2 expression. Similar complexity has been described in studies of cell-cycle and apoptosis-associated proteins in cervical lesions [9,10].

The inverse Bcl-2 coefficient in the exploratory invasive-SCC model is consistent with the group means but should not be interpreted as a protective causal effect. The model distinguishes invasive SCC from a comparison group that includes a large number of Bcl-2-rich HSILs. Consequently, the odds ratio reflects contrast between disease stages, not an intervention target. p53 and Ki-67 dominated the model because invasion in this dataset was characterized by combined proliferative acceleration and tumor-suppressor-pathway disturbance. TUNEL contributed little after those markers were included, further indicating that general cell turnover was less specific for invasion. The apparent model AUC of 0.978 is likely optimistic. It was measured in the derivation dataset, without external validation or reported cross-validation. Penalized regression reduces coefficient instability but does not itself validate a model. In addition, conventional uncertainty intervals were unavailable. The model is therefore best considered a biological summary of the cohort rather than a deployable clinical prediction tool [15-17].

The TMA results are encouraging for research economy. Moderate rank correlations indicate that cores often preserved the relative ordering of marker expression observed on conventional sections. Ki-67 showed the highest immunohistochemical correlation, plausibly because widespread proliferative activity in advanced lesions is easier to capture than spatially variable Bcl-2 expression. Using TMA can reduce antibody consumption, preserve archival tissue and allow cases to be stained under more comparable technical conditions [12,13].

Nevertheless, the term validation must be used carefully. Spearman correlation measures monotonic association; it does not measure absolute agreement, systematic bias or clinically acceptable error. Two methods can correlate strongly while producing materially different values. A robust platform-comparison study would report paired plots, intraclass correlation where appropriate, mean or median paired differences, Bland-Altman limits, core-loss rates and agreement at clinically relevant thresholds [14]. The perfect TUNEL rank correlation is particularly vulnerable to overinterpretation because the same four-category ordinal scale was used on both platforms.

TMA should therefore remain a research and quality-assurance tool rather than a substitute for conventional cervical sections. Whole sections are required to evaluate lesion architecture, epithelial maturation, glandular extension, stromal response, necrosis, margins and microinvasion. Even a technically excellent core may omit the only focus that establishes invasion. This distinction is critical in settings where the pressure to conserve reagents could otherwise encourage inappropriate diagnostic substitution.

The study is relevant to low-resource pathology systems. Full-section staining of several biomarkers in hundreds of cases is expensive and vulnerable to interrupted supply chains. A carefully constructed TMA can make local validation, training and quality-assurance projects feasible. The most practical workflow is to establish the diagnosis on H&E whole sections, select representative blocks and cores for research, use stringent controls and return to the donor section whenever a TMA result is unexpected or heterogeneous. Standardized fixation, antigen retrieval and scoring remain essential [18-20].

Strengths of the study include a four-level histological spectrum, consensus diagnosis, complementary apoptosis measures and a paired platform comparison. The analysis also separated biological interpretation from diagnostic replacement, which is particularly important for TMA research.

Several limitations constrain inference. The cross-sectional design did not capture progression from HSIL to invasive SCC, and the referral-enriched cohort limits generalizability to screening populations. Apoptosis and TUNEL were measured on coarse ordinal scales, and interobserver reproducibility was not evaluated. The paired TMA subset included 60 cases and may not represent the full range of tissue heterogeneity. Platform comparison was limited to rank correlation; agreement and systematic bias were not assessed. The ridge model lacked independent validation and uncertainty intervals.

5. CONCLUSION

Morphological apoptosis and TUNEL were higher in HSIL and invasive SCC than in benign and low-grade cervical lesions, consistent with a high-turnover neoplastic phenotype rather than effective regression. Bcl-2 expression peaked in HSIL and declined in invasive SCC, identifying a nonlinear transition in survival biology. Biopsy-TMA rank correlations support economical research use of TMA, especially for broadly expressed markers, but do not demonstrate diagnostic interchangeability. Whole-section morphology remains mandatory, and future studies should incorporate predefined scoring, detailed technical reporting, formal agreement statistics and independent validation.

DECLARATIONS

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Data availability: Aggregate data supporting the findings are reported in the article. Access to de-identified individual-level data is subject to institutional approval and participant-confidentiality requirements.

Author contributions: Thuraya Abdelah Mohammed Elhassan: Conceptualization, investigation, data curation, methodology, formal analysis and writing - original draft. Mubarak Elsaeed Elkarsani: Conceptualization, methodology, supervision, validation and writing - review and editing. Mohamed Elfatih Abdalla Abdellatif Abdelwadoud: Methodology, co-supervision, pathology interpretation, validation and writing - review and editing. Mutaz Mohamed Ibrahim Ali: Manuscript development, scientific interpretation, writing - review and editing, project administration and correspondence.

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