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Molecular-Cytological Associations Using Liquid-Based Cytology and High-Risk Human Papillomavirus Genotyping in Sudan

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Abstract:

Background: *Cervical cancer prevention in Sudan is constrained by limited organized screening, uneven laboratory capacity and conflict-related disruption. Integrated liquid-based cytology (LBC) and high-risk human papillomavirus (hrHPV) testing may provide a practical one-specimen pathway for identifying women at greatest risk.*

Objective: *To evaluate specimen and molecular workflow performance, describe cytological and hrHPV genotype patterns, and assess the relationship of hrHPV positivity with Bethesda severity and CIN2+ histology in a post-war Sudanese clinical cohort.*

Methods: *A descriptive analytical cross-sectional study included 200 women recruited through functioning health-service clusters. LBC was reported to be using the Bethesda System. Residual material underwent DNA extraction, beta-globin internal-control testing, consensus HPV PCR using MY09/MY11, GP5+/GP6+ or SPF10, and genotype determination by Sanger sequencing, targeted next-generation sequencing or other confirmatory methods. Categorical associations were assessed by chi-square tests.*

Results: *Cytology was satisfactory in 188 women (94.0%), beta-globin amplified in 195 (97.5%), and only two HPV results were invalid (1.0%). Abnormal cytology occurred in 62 women (31.0%), hrHPV positivity in 65 (32.5%), and CIN2+ in 31 (15.5%). HPV31, HPV45, HPV16 and HPV18 were the leading genotypes. hrHPV positivity was strongly associated with Bethesda category (χ^2=83.15, p<0.001) and CIN2+ histology (χ^2=46.95, p<0.001).*

Conclusion: *Combined LBC and hrHPV testing was technically feasible and clinically informative despite post-war service disruption. The substantial non-16/18 genotype burden supports broad, genotype-aware surveillance, while selective histological verification limits formal diagnostic-accuracy claims.*

Keywords: cervical cancer; liquid-based cytology; high-risk human papillomavirus; HPV genotyping; Bethesda System; CIN2+; Sudan; post-war health services

1. INTRODUCTION

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Cervical cancer remains a major example of preventable cancer inequity. Persistent infection with carcinogenic human papillomavirus is the necessary causal event for most cases, yet the clinical outcome of infection depends heavily on whether women can access vaccination, sensitive screening, diagnostic triage and treatment before invasion occurs (Bosch et al. 2002; Schiffman et al. 2007; World Health Organization 2020). The greatest burden occurs in low- and middle-income countries where these linked services are incomplete or unevenly distributed (Bray et al. 2024; Bruni et al. 2022).

Sudan combines a substantial cervical cancer burden with limited organized screening, concentrated pathology services and major conflict-related disruption. National and international summaries have emphasized low prevention coverage, fragile referral pathways and the need for locally feasible molecular and cytological models (Husain et al. 2022; ICO/IARC Information Centre on HPV and Cancer 2023). During war and early recovery, women may move between states, attend whichever facilities remain operational and lose continuity of previous screening records. A single-center design can therefore miss the dispersed population that actually reaches care.

Validated HPV testing has higher sensitivity for CIN2+ than cytology alone and provides strong reassurance after a negative result, but its lower specificity for clinically important disease requires triage (World Health Organization 2021; Perkins et al. 2020). Liquid-based cytology remains valuable because it standardizes slide preparation and retains residual material for reflex molecular testing. This one-specimen architecture is particularly attractive where recall is difficult, although its success depends on transformation-zone sampling, transport, slide quality, internal controls and contamination prevention (Arbyn et al. 2008; Nayar and Wilbur 2015).

Genotyping adds further clinical and epidemiological information because carcinogenic HPV types differ in persistence and cancer attribution. HPV16 and HPV18 dominate globally, but African studies demonstrate important contributions from HPV35, HPV45, HPV52 and HPV58 and marked regional heterogeneity (de Sanjosé et al. 2010; Denny et al. 2014; Ogembo et al. 2015; Seyoum et al. 2022). Sudanese data remain limited, and a recent local molecular study suggested that genotype patterns may not mirror global averages exactly (Ahmed et al. 2025).

The present study evaluated an integrated LBC-molecular workflow in a post-war Sudanese cluster network. The objectives were to quantify technical adequacy, describe Bethesda and hrHPV genotype distributions, and test whether consensus hrHPV positivity was associated with increasing cytological severity and CIN2+ histology. The operational hypothesis was that decentralized collection could still yield high-quality cytology and amplifiable DNA, and that hrHPV positivity would concentrate in higher-grade lesions.

2. MATERIALS AND METHODS

A descriptive analytical cross-sectional design was used to evaluate cytological, molecular and histological findings at the time of assessment. Reporting followed the main principles of the STROBE statement for observational studies (von Elm et al. 2007). This manuscript reports the molecular-cytological domain of a broader doctoral investigation; hormonal and micronutrient associations are analyzed separately to avoid duplication of outcome tables and interpretation.

Data collection was completed during the post-war or early-recovery period in Sudan, when facilities were functioning unevenly and patient movement had changed

because of displacement. Functioning cervical assessment points were grouped into four operational clusters: Soba, other hospitals or clinics, health centers and private clinics. Eligible women were recruited consecutively according to patient flow. Residence extended across Khartoum, Omdurman, Bahri, Gezira and River Nile. The design improved feasibility and geographic reach but did not constitute a probability sample of Sudanese women.

Women aged 18 years or older were eligible when they provided informed consent, underwent LBC sampling and had sufficient clinical and laboratory information. Women with previous total hysterectomy, current treatment for established invasive cervical cancer, inability to consent, an insufficient minimum dataset or heavy active bleeding that prevented interpretable sampling were excluded. The achieved sample was 200 women; the original target was larger, but conflict-related displacement, reduced attendance and interrupted follow-up constrained recruitment.

Trained clinicians sampled the transformation zone using a cervical brush, broom-type device or spatula-plus-brush technique. The collection device was rinsed or detached into LBC preservative, labelled with a coded study identifier and transported under ambient or cooled conditions according to cluster capacity. Time to laboratory receipt was recorded. Slides were prepared using the available standardized or locally validated LBC workflow, stained by the Papanicolaou method and reported according to the Bethesda System. Adequacy, transformation-zone representation, inflammation, obscuring material, organisms and diagnostic category were recorded. Equivocal and abnormal cases underwent senior review where feasible.

Residual LBC material was aliquoted in a contamination-controlled area. DNA was extracted by silica-column kits, magnetic beads, phenol-chloroform or another validated method according to availability. DNA concentration and A260/280 purity were measured. Beta-globin amplification served as human internal control. Broad HPV detection used MY09/MY11, GP5+/GP6+ or SPF10 consensus primer systems, each of which has different amplicon and type-detection characteristics (Manos et al. 1989; de Roda Husman et al. 1995; Kleter et al. 1998). Positive, negative and invalid results were recorded. HPV-positive samples were genotyped by Sanger sequencing, targeted next-generation sequencing or other confirmatory approaches. Multiple infections were counted by genotype occurrence; genotype percentages were therefore not mutually exclusive.

Colposcopy and biopsy were performed selectively according to cytology, HPV result, clinical suspicion and service availability. Histology was classified as normal, CIN1, CIN2, CIN3 or invasive cancer. CIN2+ comprises CIN2, CIN3 and invasive cancer. Treatment and follow-up outcomes were recorded where available. Because verification was selective rather than universal, the study was not designed to estimate unbiased sensitivity, specificity or predictive values.

Categorical variables were summarized as frequencies and percentages. Continuous variables were summarized by mean and standard deviation, median and interquartile range, and range. Abnormal cytology includes ASC-US, LSIL, HSIL, AGC and SCC. Pearson chi-square tests evaluated consensus HPV result versus Bethesda category and versus CIN2+ histology. Statistical significance was defined as $p<0.05$. The analysis was exploratory and individual-level because complete design variables were not available for weighted cluster analysis.

Ethical considerations

Ethical approval was obtained or maintained through the relevant institutional and clinical authorities. Participants provided informed consent. Coded identifiers protected confidentiality, and women with HSIL, suspicious lesions or invasive cancer were referred for appropriate management.

3. RESULTS

3.1 Cohort and service context

The 200 participants had a mean age of 40.3±12.1 years. Most were married, low or middle socioeconomic strata predominated, 34.5% reported displacement and 58.5% had never undergone previous cervical screening. Recruitment was distributed across four functioning service clusters, with Soba contributing 44.0% (Table 1; Figure 1). Two-thirds of women were asymptomatic, confirming that clinically important cervical abnormalities cannot be identified reliably by symptom-based attendance alone.

Table 1. Selected participant and post-war service characteristics (n=200).

Domain	Indicator	Value
Age	Mean ± SD	40.3 ± 12.1 years
Marital status	Married	145 (72.5%)
Socioeconomic status	Low or middle	162 (81.0%)
Displacement	Yes	69 (34.5%)
Previous cervical screening	Never	117 (58.5%)
Symptoms	No symptoms	133 (66.5%)
Recruitment site	Soba	88 (44.0%)
Recruitment site	Other hospital/clinic	63 (31.5%)
Recruitment site	Health center	27 (13.5%)
Recruitment site	Private clinic	22 (11.0%)

Figure 1. Distribution of participants across functioning post-war health-service clusters.

3.2 Specimen and molecular workflow performance

The LBC and molecular workflow performed well under decentralized collection conditions. Cytology was satisfactory in 188 women (94.0%), the transformation-zone component was present in 143 (71.5%), median time to the laboratory was 7.0 hours, and beta-globin amplified in 195 samples (97.5%). Only two consensus HPV results were invalid (1.0%). Ambient transport accounted for 72.5% of specimens, suggesting that the preservative and documented transport intervals supported acceptable performance even where cooling was unavailable (Table 2; Figure 2).

Table 2. Technical, cytological, molecular and histological profile of the cohort.

Domain	Category/measure	Value
Transport	Ambient	145 (72.5%)
Specimen adequacy	Satisfactory	188 (94.0%)
Transformation zone	Present	143 (71.5%)
Time to laboratory	Mean ± SD	7.2 ± 3.9 hours
Beta-globin internal control	Positive	195 (97.5%)
Consensus HPV result	Positive	65 (32.5%)
Consensus HPV result	Negative	133 (66.5%)
Consensus HPV result	Invalid	2 (1.0%)
Bethesda category	NILM	138 (69.0%)
Bethesda category	ASC-US	5 (2.5%)
Bethesda category	LSIL	24 (12.0%)
Bethesda category	HSIL	29 (14.5%)
Bethesda category	AGC	2 (1.0%)
Bethesda category	SCC	2 (1.0%)
Histology	CIN2+	31 (15.5%)
Follow-up	Completed	116 (58.0%)

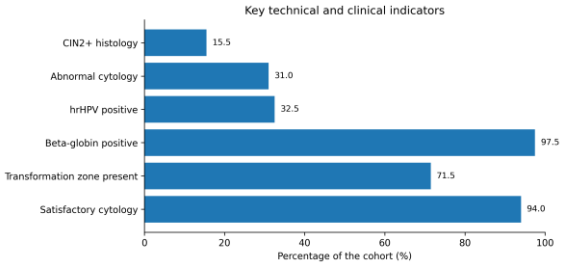

Figure 2. Key technical and clinical indicators demonstrating the integrated LBC-molecular pathway

3.3 hrHPV prevalence and genotype distribution

Consensus hrHPV positivity was detected in 65 women (32.5%). HPV31 was the most frequent genotype occurrence, followed by HPV45, HPV16 and HPV18. HPV35 and HPV33 also made meaningful contributions, while HPV58 and HPV52 were less frequent (Table 3; Figure 3). Because multiple infections were counted per genotype occurrence, percentages were not expected to sum to 100%.

Table 3. Distribution of detected hrHPV genotypes among consensus HPV-positive women (n=65).

Genotype	Frequency	% of HPV-positive women
HPV31	16	24.6
HPV45	15	23.1
HPV16	14	21.5
HPV18	14	21.5
HPV35	9	13.8
HPV33	7	10.8
HPV58	2	3.1
HPV52	1	1.5

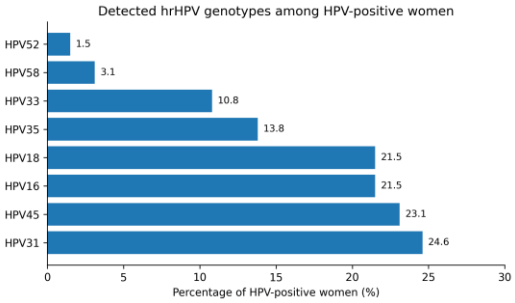

Figure 3. hrHPV genotype occurrences among consensus HPV-positive women.

3.4 Association of hrHPV with cytological and histological severity

Consensus HPV result was strongly associated with Bethesda category (χ^2=83.15, df=5, p<0.001). HPV-negative women were concentrated in NILM, whereas HPV-positive women were disproportionately represented in LSIL, HSIL, AGC and SCC. Among HPV-positive women, 38.5% had HSIL compared with 3.0% of HPV-negative women (Table 4, Panel A; Figure 4).

A similarly strong relationship was observed with CIN2+ histology (χ^2=46.95, df=1, p<0.001). CIN2+ was recorded in 27 of 65 HPV-positive women (41.5%) but in only four of 135 women classified as HPV negative in the analytical cross-tabulation (3.0%) (Table 4, Panel B). This contrast is clinically important, although selective biopsy and classification of unverified women within the non-CIN2+ group introduce verification bias.

Table 4. Consensus HPV result in relation to Bethesda category and CIN2+ histology.

Panel A. Bethesda category by consensus HPV result.

HPV result	NILM	ASC-US	LSIL	HSIL	AGC	SCC
Negative	120 (88.9%)	3 (2.2%)	8 (5.9%)	4 (3.0%)	0	0
Positive	18 (27.7%)	2 (3.1%)	16 (24.6%)	25 (38.5%)	2 (3.1%)	2 (3.1%)

χ^2=83.15, df=5, p<0.001. Row percentages are shown.

Panel B. CIN2+ histology by consensus HPV result.

HPV result	<CIN2 or not done	CIN2+
Negative	131 (97.0%)	4 (3.0%)
Positive	38 (58.5%)	27 (41.5%)

χ^2=46.95, df=1, p<0.001. Row percentages are shown.

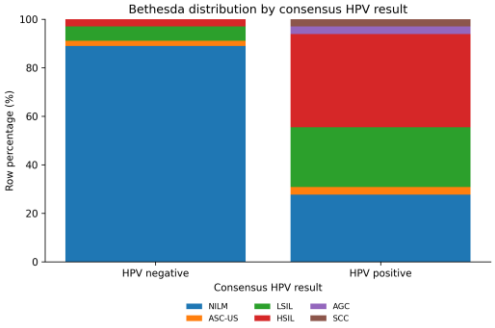

Figure 4. Cytological severity distribution according to consensus HPV result.

4. DISCUSSION

The study generated three principal findings. First, decentralized post-war collection produced high cytology adequacy, strong beta-globin internal-control performance and few invalid molecular results. Second, the clinic-enriched cohort carried a substantial burden of abnormal cytology and hrHPV, with a genotype pattern extending well beyond HPV16 and HPV18. Third, hrHPV positivity showed a strong biological gradient with Bethesda severity and CIN2+ histology. These findings jointly support the feasibility and clinical relevance of an integrated LBC-HPV pathway, while the design does not support population-prevalence or formal diagnostic-accuracy claims.

A 94.0% satisfactory cytology rate and 97.5% beta-globin positivity are important in a context where collection occurred across several service entry points. The result indicates that standardized forms, trained sampling, immediate placement in preservative, coded transport and laboratory quality control were able to preserve diagnostic material. Arbyn et al. (2008) found that LBC reduces unsatisfactory preparations, but LBC itself cannot compensate for poor transformation-zone sampling or delayed processing. The 71.5% transformation-zone representation therefore identifies a practical improvement target, especially among older women in whom the squamocolumnar junction may be endocervical.

The use of three consensus primer systems broadened analytical access when laboratory resources varied, but it also introduced methodological heterogeneity. MY09/MY11, GP5+/GP6+ and SPF10 differ in amplicon length and type-specific sensitivity (Manos et al. 1989; de Roda Husman et al. 1995; Kleter et al. 1998). A future national pathway should preferably standardize one clinically validated assay, use external quality assurance and reserve sequencing for genotyping or discordant samples. The present finding demonstrates operational feasibility, not interchangeability of all methods.

Abnormal cytology was identified in 31.0% of participants, with HSIL slightly more frequent than LSIL. Such a distribution would be unusual in an unselected screening population. It is best explained by intentional enrichment of women attending for follow-up, suspicious lesions or previous abnormalities, combined with weak prior screening coverage. The low ASC-US frequency may reflect experienced review, threshold differences or selective recruitment of clearer abnormalities. Bethesda categories are standardized, but equivocal thresholds remain vulnerable to interobserver variation (Solomon et al. 2002; Nayar and Wilbur 2015).

HPV31 and HPV45 numerically exceeded HPV16 and HPV18, while HPV35 and HPV33 also contributed. The ranking should be interpreted cautiously because only 65 women were HPV positive, multiple infections were counted and genotyping methods were heterogeneous. Nevertheless, the pattern is biologically credible. Worldwide attribution studies identify all of these types as carcinogenic, and African evidence repeatedly shows a meaningful contribution from HPV35 and HPV45 (de Sanjosé et al. 2010; Denny et al. 2014; Ogembo et al. 2015). The finding does not diminish the importance of HPV16/18 vaccination; rather, it supports broad hrHPV detection and genotype surveillance capable of monitoring non-16/18 disease.

The local result also aligns with the wider observation that genotype distribution varies by geography, HIV prevalence, lesion grade and assay platform (Seyoum et al. 2022). Before altering screening or vaccine policy, the pattern should be confirmed in larger population-based and histology-stratified studies using a

standardized genotyping method. In the interim, Sudanese screening should avoid assays or triage strategies that are so narrow that they overlook clinically important African genotypes.

The association between hrHPV and Bethesda severity is the most internally coherent result. HPV-positive women were distributed across LSIL, HSIL, AGC and SCC, whereas HPV-negative women were overwhelmingly NILM. This is consistent with the causal model in which HPV infection precedes and drives most squamous precancer (Bosch et al. 2002; Schiffman et al. 2007). The magnitude and direction of the gradient provide stronger evidence than the p value alone.

The relationship with CIN2+ was also large, but its interpretation is restricted by selective verification. Only 43 women underwent biopsy, and referral was likely influenced by cytology, HPV or clinical suspicion. Most women without histology were included in the analytical non-CIN2+ category, which can inflate apparent separation and prevents unbiased estimates of sensitivity, specificity, positive predictive value or negative predictive value. A future verification study should apply an appropriate reference standard to all participants or use statistically valid correction for partial verification.

The results support a pragmatic one-specimen pathway: collect LBC material, report cytology, perform reflex hrHPV testing and use cytology plus genotype to priorities colposcopy. Such a pathway reduces repeat sampling and is suited to settings where displacement and transport barriers make repeated visits difficult. However, the follow-up results show that 16.0% were lost and 21.5% remained pending. Screening benefit therefore depends on patient navigation, reliable communication, referral capacity and treatment availability, not on laboratory testing alone (World Health Organization 2021). Strengths include integration of cytology, molecular testing, genotyping and histology; recruitment across several functioning clusters; high internal-control performance; and transparent retention of invalid and not-done categories. Limitations include cross-sectional design, referral enrichment, reduced sample size, unequal cluster sizes, absence of complex-sample adjustment, heterogeneous extraction and primer systems, mixed genotyping methods, sparse cytology categories and selective biopsy. The findings are therefore most useful for pathway design and hypothesis generation rather than national prevalence estimation.

5. CONCLUSION

Liquid-based cytology combined with consensus hrHPV detection and genotype characterization was technically feasible and clinically informative in a post-war Sudanese service network. High specimen adequacy and internal-control performance demonstrate that decentralized collection can support integrated testing when procedures are standardized. hrHPV positivity was strongly concentrated in more severe Bethesda categories and CIN2+ histology, while the genotype spectrum showed substantial non-16/18 representation. The most practical next step is a prospective, standardized and cluster-aware validation study linked to complete colposcopic verification and reliable treatment follow-up.

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FUNDING

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICAL APPROVAL

Ethical approval was obtained or maintained through the relevant institutional and clinical authorities. All participants provided informed consent, confidentiality was protected through coded identifiers, and women with clinically significant abnormalities were referred for appropriate evaluation and management.

DATA AVAILABILITY

The aggregate data supporting the findings are presented in the manuscript. De-identified participant-level data may be available from the corresponding author upon reasonable request and subject to institutional and ethical approval.

AUTHOR CONTRIBUTIONS

Hala Mahmoud Elhag Mohamed conducted the doctoral investigation, participated in data and specimen collection, laboratory work, data curation and thesis drafting. Mohammed Siddig AbdElaziz Mohammed and Ali Sid Ahmed provided doctoral supervision, methodological guidance and critical review. Mutaz Mohamed Ibrahim Ali contributed conceptualization of the molecular-cytological publication domain, manuscript redesign, statistical interpretation, journal-targeted scientific editing and correspondence. All authors reviewed and approved the final manuscript.

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