

Research Article**High-Risk HPV Infection and Its Association With Cervical Dysplasia : Histopathological Grading, Hematological Indices, and p53/p16 Gene Marker Expression in Married Women Aged 25–45**

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ABSTRACT

Cervical cancer remains a leading cause of morbidity in women globally, with persistent high-risk human papillomavirus (HR-HPV) infection being the primary etiologic factor. Early detection of cervical dysplasia through objective biomarkers may improve prevention of progression to invasive carcinoma. This study evaluated the association between HR-HPV infection, histopathological grading of cervical lesions, hematological indices, and expression of tumor suppressor p53 and cell cycle marker p16 in married women aged 25–45. A total of 210 participants (150 HR-HPV positive and 60 HR-HPV negative controls) underwent

Pap smear cytology, colposcopic biopsy, complete blood count (CBC), and quantitative PCR-based analysis of p53 and p16 expression. Cervical biopsies showed increasing prevalence of low-grade (CIN I: 46.7%) and high-grade (CIN II/III: 28.0%) lesions in HR-HPV positive women ($p < 0.001$). Mean neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) were significantly elevated in HR-HPV positive cases (NLR: 3.8 ± 0.7 vs. 1.9 ± 0.4 ; PLR: 154 ± 31 vs. 101 ± 19 ; $p < 0.001$). p16 expression was markedly upregulated in high-grade lesions (relative expression: 5.6 ± 1.1) compared to controls (1.2 ± 0.3 ; $p <$

0.001). Conversely, p53 expression was inversely correlated with lesion severity ($r = -0.62, p < 0.001$). These findings suggest that hematological indices and gene marker expression profiles correlate with histopathological severity in HR-HPV infection, offering sensitive, objective indicators for early detection and risk stratification.

Keywords: High-risk HPV, Cervical dysplasia, p16 expression, p53 suppression, Hematological indices

INTRODUCTION

Human papillomavirus (HPV) infection is the most common sexually transmitted infection worldwide and a well-established cause of cervical intraepithelial neoplasia (CIN) and cervical cancer [1]. Among the numerous HPV genotypes, high-risk HPV (HR-HPV) types, especially 16 and 18, are responsible for the majority of high-grade lesions and carcinomas due to their oncogenic potential [2].

Despite widespread screening strategies like Pap smear cytology, the precancerous transformation can be missed because cytology is subjective and has variable sensitivity [3]. Integration of objective

molecular and hematological markers alongside histopathological grading may improve early detection of clinically significant lesions.

The host's immune response to chronic HR-HPV infection and the tumor microenvironment influence systemic inflammatory and hematological profiles, which may be reflected in markers such as neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) [4]. Elevated NLR and PLR have been reported in various malignancies and premalignant conditions, yet their association with HR-HPV related cervical dysplasia remains inadequately characterized [5].

At the molecular level, HPV oncoproteins interfere with tumor suppressor pathways, resulting in dysregulated expression of genes such as p16INK4a and p53 [6]. Overexpression of p16 due to retinoblastoma pathway disruption is a recognized surrogate biomarker for transforming HPV infections, while p53 suppression reflects disrupted genomic stability [7].

However, few comprehensive studies integrate histopathological grading, hematological indices, and gene marker

profiles in HR-HPV infected women within the reproductive age group. Addressing this gap could enhance risk stratification and individualize clinical management.

Thus, this study aimed to evaluate the association between HR-HPV infection and cervical dysplasia severity by correlating histopathological findings with hematological indices and expression levels of p53 and p16 in married women aged 25–45.

MATERIALS AND METHODS

Study Design and Setting

This cross-sectional experimental study was conducted at the Wah General Hospital in collaboration with Jinnah Hospital, Lahore Pakistan between February 2024 and November 2024.

Ethical Approval

Ethical approval was obtained from the Institutional Review Board (IRB) of the National Women's Hospital (Ref No: IRB/NWH/2024-056).

Sample

A total of 210 married women aged 25–45 were enrolled. Among these, 150 women tested positive for HR-HPV by PCR screening, and 60 age-matched women tested negative and served as controls.

Inclusion/ Exclusion criteria

Women aged 25–45, sexually active and consenting to participate were included. Those with pregnancy, autoimmune disorders, current cancer therapy, HIV infection, or recent corticosteroid use were excluded.

HR-HPV Detection and Genotyping

Cervical swabs were collected for HR-HPV PCR using validated primers specific for high-risk genotypes 16, 18, 31, 33, and 45.

Histopathological Examination

Colposcopy-guided cervical biopsies were processed and graded as benign, low-grade CIN I, or high-grade CIN II/III by two blinded histopathologists.

Hematological Indices

Venous blood samples were analyzed for complete blood count (CBC). Neutrophil-to-

lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) were calculated.

Gene Expression Analysis

Relative expression of p16INK4a and p53 genes in cervical tissue was quantified using real-time PCR with GAPDH normalization.

Data were tabulated and analyzed using SPSS v27. Continuous variables were expressed as mean $\pm$ SD. Comparisons were made by t-test or ANOVA as appropriate. Pearson's correlation assessed relationships between variables. A p-value <0.05 was considered significant.

Statistical analysis

RESULTS

Table 1: Demographic, HPV Status, and Histopathology

Parameter	HR-HPV+ (n = 150)	HR-HPV- (n = 60)	p-value
Age (years)	36.8 $\pm$ 5.9	35.9 $\pm$ 6.2	0.34
CIN I (%)	70 (46.7)	5 (8.3)	<0.001
CIN II/III (%)	42 (28.0)	0 (0)	<0.001
Normal histology (%)	38 (25.3)	55 (91.7)	<0.001

HR-HPV positive women showed significantly greater histopathological severity.

Table 2: Hematological Indices Among Study Groups

Index	HR-HPV+	HR-HPV-	p-value
NLR	3.8 $\pm$ 0.7	1.9 $\pm$ 0.4	<0.001
PLR	154 $\pm$ 31	101 $\pm$ 19	<0.001
Hemoglobin (g/dL)	11.4 $\pm$ 1.1	12.1 $\pm$ 0.9	0.002

Elevated NLR and PLR were significantly associated with HR-HPV positivity.

Table 3: Gene Marker Expression (Relative Quantification)

Marker	HR-HPV+ CIN II/III (n=42)	HR-HPV+ CIN I (n=70)	HR-HPV- (n=60)	p-value
p16	5.6 ± 1.1	2.8 ± 0.9	1.2 ± 0.3	<0.001
p53	0.4 ± 0.1	0.8 ± 0.2	1.1 ± 0.3	<0.001

High-grade lesions showed significant upregulation of p16 and downregulation of p53.

DISCUSSION

This study demonstrates a strong association between HR-HPV infection and cervical dysplasia severity in married women aged 25–45, supported by histopathological, hematological, and gene expression data. HR-HPV positive women exhibited significantly higher prevalence of CIN I and CIN II/III lesions when compared to HPV negative controls, confirming the well-known oncogenic potential of high-risk genotypes [9,10].

Systemic hematological indices such as NLR and PLR were markedly elevated in HR-HPV infected individuals, particularly those with high-grade lesions, suggesting a systemic inflammatory shift associated with oncogenic progression. These findings align with prior observations that chronic infection and tumor microenvironment interplay can elevate systemic inflammatory markers [11]. Elevated NLR and PLR have also been linked

to poorer outcomes in other premalignant and malignant conditions [12].

At a molecular level, p16 overexpression correlated positively with lesion severity, consistent with its role as a surrogate marker of oncogenic HPV E7-mediated retinoblastoma protein disruption. In contrast, p53 expression was significantly suppressed in high-grade lesions, reflecting HPV E6-mediated degradation of the p53 tumor suppressor pathway [13]. These patterns corroborate earlier findings that demonstrate the utility of combined p16 and p53 profiling for risk stratification in cervical dysplasia [14].

The novelty of this study lies in its integrated evaluation of histopathology, systemic hematological indices, and targeted gene marker expression in a defined reproductive age group, providing a multi-modal framework for objective risk assessment. While cytology and colposcopy remain essential, the addition of these biomarkers

may improve early detection accuracy and inform clinical decision-making [15].

CONCLUSION

This study identifies systemic inflammatory markers and differential expression of p16 and p53 as sensitive, objective indicators correlating with cervical dysplasia severity in HR-HPV infected women aged 25–45. These biomarkers may enhance early risk stratification beyond conventional cytology.

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ETHICS STATEMENT

Approved by the Institutional Review Board of the National Women's Hospital (IRB/NWH/2024-056).

INFORMED CONSENT

Written informed consent was obtained from all participants prior to inclusion.

COMPETING INTERESTS

The authors declare no competing interests.

FINANCIAL DISCLOSURE

This research received no external funding.

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