Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(4), 2742-2747 | ISSN:2251-6727

Ki-67 as a Biomarker in the Progression of Cervical Lesions: A Clinicopathological Correlation

Vinita Raj, Nausheen Sanaullah Khan, Priyanka Singh, Javed Iqbal, Kusum Lata

[1]Department of Pathology, [2]Department of Obstetrics & Gynaecology, Integral Institute of Medical Sciences and Research, Lucknow, Uttar Pradesh, India.

Corresponding author:

Prof. (Dr.) Nausheen Sanaullah Khan,

Department of Pathology, Integral Institute of Medical Sciences and Research, Lucknow, Uttar Pradesh, India

(Received: 16 June 2025 Revised: 20 July 2025 Accepted: 19 August 2025)

KEYWORDS

Ki-67; Cervical lesions; CIN; Squamous cell carcinoma; Immunohistochemistry

ABSTRACT:

Introduction: Cervical carcinogenesis follows a spectrum from normal epithelium to cervical intraepithelial neoplasia (CIN) and eventually invasive carcinoma. Accurate grading of these lesions is critical for clinical management. Ki-67, a nuclear protein expressed during active cell cycle phases, reflects cellular proliferation and may serve as an effective marker for lesion severity.

Methods: A total of 65 cervical biopsy and hysterectomy specimens (CIN 1, 2, 3, and squamous cell carcinoma) were collected over a 2-year period. Routine histopathological examination was followed by immunohistochemical staining using anti-Ki-67 (clone MIB-1). Ki-67 expression was graded: score 0 (no staining), 1 (<10%), 2 (10–50%), and 3 (>50%). Scores 2 and 3 were considered positive. Correlations between Ki-67 expression and lesion grade were analyzed.

Results: Ki-67 expression showed a statistically significant correlation with lesion severity ($p < 0.0001$). CIN-1 and CIN-2 lesions demonstrated absent or low proliferation, while CIN-3 showed moderate Ki-67 expression. Squamous cell carcinoma exhibited the highest Ki-67 positivity with 72.2% (n=13) showing 10–50% expression and 27.8% (n=5) >50%. Ki-67 demonstrated a sensitivity of 67%, specificity of 96%, and overall accuracy of 88% in distinguishing malignant from premalignant lesions.

Conclusions: Ki-67 immunohistochemistry is a useful adjunct in grading cervical lesions. Its progressive increase across lesion stages reinforces its value as a diagnostic biomarker for assessing cellular proliferation and malignant transformation in cervical neoplasia.

1. Introduction

Cervical cancer is one of the leading causes of cancer-related morbidity and mortality in women globally, particularly in low- and middle-income countries (LMICs), where effective screening and vaccination programs are often lacking [1]. In India, cervical cancer remains the second most common cancer among women, accounting for nearly 20% of the global burden, with an estimated 123,907 new cases and 77,348 deaths annually [2, 3]. The development of cervical cancer is well understood to follow a gradual histopathological continuum from normal squamous epithelium through cervical intraepithelial neoplasia (CIN grades 1 to 3) to invasive squamous cell carcinoma (SCC) [4].

Although the Papanicolaou (Pap) smear and histopathological biopsy remain the cornerstone of cervical cancer screening and diagnosis, several limitations exist. These include sampling errors, subjective interpretation, interobserver variability, and difficulties in distinguishing high-grade lesions from inflammatory or regenerative changes [5,6, 7]. Consequently, adjunctive biomarkers that provide objective and reproducible diagnostic support have become an area of active investigation.

Ki-67 is a well-characterized nuclear protein that is expressed exclusively during the active phases of the cell cycle (G1, S, G2, and M), but not in resting (G0) cells, making it a reliable marker of cellular proliferation [8].

Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(4), 2742-2747 | ISSN:2251-6727

In normal cervical epithelium, Ki-67 expression is typically restricted to the basal and parabasal layers. However, its expression extends to the upper epithelial layers in dysplastic and neoplastic conditions, reflecting increased proliferative activity [9, 10, 11]. Multiple studies have demonstrated a progressive increase in Ki-67 expression across the CIN spectrum, with the highest levels observed in invasive squamous cell carcinoma (12). This graded expression pattern suggests that Ki-67 may serve as a surrogate marker for lesion severity and an aid in differentiating between low-grade and high-grade intraepithelial neoplasia. In particular, Ki-67 has been shown to correlate with histological grade, HPV status, and clinical outcome, enhancing its utility in cervical cancer screening algorithms (11, 13).

Given the rising incidence of cervical neoplasia and the limitations of traditional histological evaluation, the current study was designed to evaluate the immunohistochemical expression of Ki-67 in premalignant (CIN 1, CIN 2, CIN 3) and malignant (SCC) cervical lesions. The study also aims to correlate Ki-67 expression with histological grade and clinicopathological parameters to assess its potential as a diagnostic and prognostic biomarker.

2. Materials and Methods

Study Design and Setting

This observational study was conducted in the Department of Pathology at Integral Institute of Medical Sciences and Research (IIMSR), Lucknow, spanning a two-year period from April 2023 to March 2025. A total of 65 cervical tissue specimens—either cervical biopsies or hysterectomy samples—were included. These samples were obtained from patients presenting to the Department of Obstetrics and Gynaecology and were histologically diagnosed as either premalignant [Cervical Intraepithelial Neoplasia (CIN grades 1–3)] or malignant (Squamous Cell Carcinoma) lesions. Inclusion criteria were: availability of a confirmed histopathological diagnosis and adequate tissue for immunohistochemical analysis. Exclusion criteria comprised insufficient tissue samples, cases with prior chemotherapy or radiotherapy, and patients diagnosed with other concurrent malignancies. The study was reviewed and approved by the Institutional Ethics Committee, and informed written consent was obtained from all participants.

Histopathological Evaluation and Immunohistochemistry:

All collected tissue samples were fixed in 10% neutral buffered formalin, routinely processed, and embedded in paraffin blocks. Sections of 3–5 µm thickness were cut and stained with hematoxylin and eosin for microscopic evaluation and histological grading. For immunohistochemical detection of Ki-67, sections were deparaffinized, rehydrated through graded alcohols, and subjected to antigen retrieval using Tris-EDTA buffer (pH 9.0) in a pressure cooker at 110°C for 20 minutes. Endogenous peroxidase activity was blocked using 3% hydrogen peroxide. The sections were then incubated with a mouse monoclonal anti-Ki-67 antibody (Clone MIB1, Biogenex), followed by treatment with a secondary antibody and visualization using the DAB (3,3′-diaminobenzidine) chromogen. Hematoxylin was used as the nuclear counterstain.

Human tonsil tissue served as the positive control for Ki-67 staining, while negative controls were processed without the primary antibody to confirm staining specificity. Slides were examined independently by a panel of experienced pathologists. The evaluation of Ki-67 nuclear expression was performed on immunohistochemically stained tissue sections using high-power magnification (×400). The scoring was conducted in a semi-quantitative manner based on the proportion of epithelial cell nuclei showing positive immunoreactivity for Ki-67. The scoring system was categorized into four levels. Score 0 indicated the complete absence of nuclear staining, representing no detectable proliferative activity within the examined field. Score 1 was assigned when less than 10% of the epithelial nuclei showed positive staining, reflecting low proliferative activity. Score 2 corresponded to moderate proliferative activity, where between 10% and 50% of the nuclei were positively stained. Finally, score 3 indicated high proliferative activity, with more than 50% of epithelial nuclei showing strong nuclear positivity for Ki-67. For the purpose of statistical and diagnostic interpretation, scores of 2 and 3 were considered Ki-67 positive, signifying increased cellular proliferation and a higher likelihood of disease progression. This scoring method allowed for standardized assessment across lesion types and facilitated correlation with histological grade and clinical behavior.

Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(4), 2742-2747 | ISSN:2251-6727

Statistical Analysis:

All data were compiled and analyzed using SPSS software version 21.0 (IBM, Chicago, IL, USA). Categorical variables were expressed as frequencies and percentages, while continuous variables were summarized using mean ± standard deviation. The association between Ki-67 expression and lesion category (premalignant vs malignant) was assessed using the Chi-square test or Fisher's exact test, as appropriate. Diagnostic parameters such as sensitivity, specificity, and accuracy were calculated using 2×2 contingency tables. A p-value of <0.05 was considered statistically significant.

3. Results

A total of 65 cervical lesions were analyzed, comprising both premalignant (n=47) and malignant (n=18) cases as presented in table 1. The distribution of lesions included 37 cases of CIN 1 (56.9%), 8 cases of CIN 2 (12.3%), 2 cases of CIN 3 (3.0%), and 18 cases of squamous cell carcinoma (SCC) (27.7%). The mean age of patients increased with lesion severity, ranging from 35.95 years in CIN 1 cases to 54.8 years in SCC cases. Ki-67 expression demonstrated a progressive increase in proliferative activity with advancing lesion grade. Among the 37 CIN 1 cases, 83.8% (n=31) showed no Ki-67 expression (Score 0), while 16.2% (n=6) showed low proliferative activity with <10% positive nuclei (Score 1). None of the CIN 1 cases exhibited moderate or high Ki-67 expression. In CIN 2, 75.0% of cases (n=6) were negative for Ki-67 (Score 0), while 25.0% (n=2) showed low positivity (<10%). Similar to CIN 1, there were no cases with moderate or high proliferative indices. However, both CIN 3 cases (100%) exhibited moderate Ki-67 expression (10–50%), reflecting increased cellular proliferation in high-grade dysplasia. In contrast, all 18 SCC cases demonstrated significant Ki-67 positivity: 72.2% (n=13) had moderate proliferative activity (10–50%), and 27.8% (n=5) showed high expression (>50%). None of the SCC cases were negative for Ki-67 staining. These findings reinforce the utility of Ki-67 as a biomarker, showing a clear trend of increasing expression from low-grade premalignant lesions to invasive carcinoma, correlating strongly with lesion severity and proliferative potential.

Comparative Analysis of Ki-67 Expression:

The comparative distribution of Ki-67 expression between premalignant and malignant cervical lesions is depicted in table 2. Among the 47 premalignant cases (comprising CIN 1, CIN 2, and CIN 3), Ki-67 expression was predominantly low. A majority, 37 cases (56.9%), exhibited no Ki-67 immunoreactivity (Score 0), while 8 cases (12.3%) showed low proliferative activity with <10% nuclear positivity (Score 1). Only 2 cases (3.0%) demonstrated moderate proliferation (10–50% positive nuclei, score 2), and none showed high expression (>50%). In contrast, all 18 malignant cases (squamous cell carcinoma) demonstrated significantly higher Ki-67 expression. 13 cases (20.0%) showed moderate nuclear positivity (Score 2), and 5 cases (7.7%) demonstrated high Ki-67 expression with >50% positive nuclei (Score 3). Notably, none of the malignant cases were negative for Ki-67, underscoring its strong association with malignant transformation. Statistical analysis revealed a highly significant difference in Ki-67 expression between premalignant and malignant groups ($p = 0.0007$, $\chi^2 = 35.17$, $df = 3$), with a Cramér's V of 0.73, indicating a strong effect size. This trend highlights a progressive increase in cellular proliferation from low-grade lesions to invasive carcinoma, supporting the utility of Ki-67 as a valuable diagnostic marker for assessing lesion severity.

Table 1: Histopathological Diagnoses, Clinical Features, and Ki-67 Expression among Study population.

Diagnosis	Lesion Type	N (%)	Mean Age	Ki-67 Score 0	Ki-67 Score <10 %	Ki-67 Score 10–50 %	Ki-67 Score >50 %
CIN-1	Premalignant	37 (56.9)	35.95	31 (83.8%)	6 (16.2%)	0	0
CIN-2	Premalignant	8 (12.3)	42.0	6 (75.0%)	2 (25.0%)	0	0

Journal of Chemical Health Risks
www.jchr.org
JCHR (2025) 15(4), 2742-2747 | ISSN:2251-6727

CIN-3	Premalignant	2 (3.0)	35.0	0	0	2 (100%)	0
SCC	Malignant	18 (27.7)	54.8	0	0	13 (72.2%)	5 (27.8%)

Table 2: Ki-67 Expression Across Premalignant and Malignant Cervical Lesions.

Analysis Category	Lesion Type	Ki-67 Score 0	Ki-67 <10 %	Ki-67 10–50%	Ki-67 >50 %	p-value
Ki-67 Expression by Lesion Type	Premalignant (n=47)	37 (56.9%)	8 (12.3%)	2 (3.0%)	0 (0%)	0.0007 (χ^2 = 35.17, df = 3, Cramér's V = 0.73)
	Malignant (n=18)	0 (0%)	0 (0%)	13 (20.0%)	5 (7.7%)	

Diagnostic Utility of Ki-67:

The diagnostic performance of Ki-67 immunohistochemistry in distinguishing between different categories of cervical lesions is summarized in table 3. When used to differentiate malignant lesions (squamous cell carcinoma) from premalignant lesions (CIN 1–3), Ki-67 showed a sensitivity of 67%, specificity of 96%, and overall diagnostic accuracy of 88%. It correctly identified 12 true positives and 45 true negatives, with 2 false positives and 6 false negatives. These findings suggest that Ki-67 is a highly specific marker for malignancy and may be useful in confirming invasive disease, though it may miss some malignant cases when used alone. When Ki-67 was evaluated for its ability to detect high-grade cervical lesions (≥ CIN 2), the biomarker demonstrated perfect specificity (100%) but moderate sensitivity of 50%, with an overall accuracy of 78%. In this context, it correctly identified 14 true positive cases and 37 true negatives, without any false positives, but with 14 false negatives. This indicates that while Ki-67 is highly specific in ruling in high-grade lesions, its limited sensitivity may result in under-detection of some cases when used as a sole marker. These results support the potential utility of Ki-67 as a reliable adjunctive tool in identifying malignant and high-grade premalignant cervical lesions, particularly when specificity is prioritized in clinical decision-making.

Table 3: Diagnostic Performance of Ki-67 in Cervical Lesions.

Diagnostic Context	Marker	Sensitivity	Specificity	Accuracy	TP	TN	FP	FN
Malignant vs. Premalignant	Ki-67	67%	96%	88%	12	45	2	6
Identifying ≥ CIN 2 Lesions	Ki-67	50%	100%	78%	14	37	0	14

4. Discussion

Cervical cancer remains one of the most significant health burdens for women globally, particularly in low- and middle-income countries where access to routine screening and early detection strategies remains limited [1]. Despite the widespread adoption of cytology-based screening methods like the Pap smear, limitations such as sampling errors, interpretive subjectivity, and interobserver variability continue to compromise diagnostic precision especially in differentiating between premalignant lesions and early invasive carcinoma [14]. Consequently, there is an increasing need for reliable and reproducible biomarkers to serve as adjuncts to histopathological assessment. Ki-67, a proliferation-associated nuclear antigen, has emerged as one of the most widely studied and promising immunohistochemical markers in this context.

Ki-67 is expressed exclusively in actively dividing cells during all phases of the cell cycle (G1, S, G2, and M) and is absent in resting (G0) cells, making it a robust indicator of proliferative activity [15]. In cervical

Journal of Chemical Health Risks
www.jchr.org
JCHR (2025) 15(4), 2742-2747 | ISSN:2251-6727

pathology, Ki-67 expression is generally restricted to the basal and parabasal layers in normal epithelium and low-grade squamous intraepithelial lesions (CIN 1). However, in high-grade lesions (CIN 2 and CIN 3) and invasive squamous cell carcinoma (SCC), Ki-67 expression expands to the upper layers of the epithelium or is diffusely expressed throughout the tumor tissue, reflecting enhanced proliferative capacity [11, 12].

In the present study, we observed a statistically significant and progressive increase in Ki-67 expression from premalignant to malignant cervical lesions ($p < 0.0007$), in concordance with previous reports (13, 16). Specifically, Ki-67 negativity (Score 0) was noted in 83.8% of CIN 1 and 75% of CIN 2 cases, indicating low proliferative activity. In contrast, both CIN 3 cases showed moderate Ki-67 expression (10–50%), and all SCC cases were positive, with 72.2% showing moderate and 27.8% showing high (>50%) Ki-67 nuclear staining. These results align with studies by Singh et al. (2023) and 16. Gahine R et al. (2025), which have emphasized that increased Ki-67 expression is significantly associated with lesion grade and serves as a key marker of transformation and malignancy [11,16].

The pattern of Ki-67 distribution is particularly valuable in cases where morphological features alone are insufficient to distinguish reactive epithelial changes from true dysplasia. Ki-67 positivity in the upper third of the epithelium or diffuse expression in invasive carcinoma supports a neoplastic process and may help to resolve diagnostic ambiguities, especially in limited biopsy material [17]. Additionally, Ki-67 complements the histological evaluation by providing quantitative insight into the lesion's biological behavior and its potential for progression.

When assessed for diagnostic utility, Ki-67 showed a sensitivity of 67% and specificity of 96% for distinguishing malignant from premalignant lesions, with an overall accuracy of 88%. These values are consistent with earlier findings by Mitildzans et al. (2017), who reported increased Ki-67 index with escalating CIN grade and invasive carcinoma [18]. In our study, for identifying high-grade lesions (≥ CIN 2), Ki-67 showed perfect specificity (100%) and an accuracy of 78%, though sensitivity remained moderate at 50%, underscoring its role as a strong confirmatory rather than screening marker. The increased expression of Ki-67 with advancing histologic severity underscores its potential not only in diagnosis but also in risk stratification. As noted by Zhong et al. (2015), a high Ki-67 index may indicate lesions more likely to progress to carcinoma, making it useful in deciding treatment thresholds in low-grade cases [12].

Furthermore, our findings support the integration of Ki-67 into standard immunohistochemical panels for cervical biopsy evaluation, especially in equivocal or borderline lesions. Several international studies have validated the clinical utility of Ki-67 when used alone or in combination with other markers such as p16 [19]. Although p16/Ki-67 dual staining is more commonly employed in clinical screening algorithms, Ki-67 alone remains a cost-effective and readily interpretable tool, particularly in resource-limited settings.

Nevertheless, the present study has limitations. The modest sample size and single-center design may affect generalizability. In addition, HPV DNA testing was not included, which limits the ability to correlate Ki-67 expression with viral oncogenic activity. Interobserver variability in scoring immunostaining and the absence of long-term follow-up further restrict prognostic evaluation.

Despite these limitations, the results reinforce the growing evidence supporting Ki-67 as a reliable and practical marker for assessing cervical epithelial proliferation and neoplastic progression. Future studies should aim for multicentric designs with larger cohorts, integration of HPV genotyping, and longitudinal follow-up to establish Ki-67's prognostic significance and its role in treatment planning.

Acknowledgements: The authors wish to acknowledge the Integral Institute of medical Sciences and Research for providing the manuscript communication number (IU/R&D/2025-MCN0003821) to this submission.

References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin. 2021;71(3):209-249. 10.3322/caac.21660

Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(4), 2742-2747 | ISSN:2251-6727

2. Bhatla N, Meena J, Kumari S, Banerjee D, Singh P, Natarajan J. Cervical Cancer Prevention Efforts in India. Indian J Gynecol Oncol. 2021;19(3):41.10.1007/s40944-021-00526-8.
3. Ramamoorthy T, Kulothungan V, Sathishkumar K, Tomy N, Mohan R, Balan S, Mathur P. Burden of cervical cancer in India: estimates of years of life lost, years lived with disability and disability adjusted life years at national and subnational levels using the National Cancer Registry Programme data. Reproductive Health. 2024;21(1):111.
4. Schiffman M, Wentzensen N. From human papillomavirus to cervical cancer. Obstet Gynecol. 2010;116(1):177–185.
5. Najib FS, Hashemi M, Shiravani Z, Poordast T, Sharifi S, Askary E. Diagnostic Accuracy of Cervical Pap Smear and Colposcopy in Detecting Premalignant and Malignant Lesions of Cervix. Indian J Surg Oncol. 2020;11(3):453-458. doi: 10.1007/s13193-020-01118-2.
6. Nessa A, Nahar KN, Begum SA, Anwary SA, Hossain F, Nahar K. Comparison between visual inspection of cervix and cytology-based screening procedures in Bangladesh. Asian Pac J Cancer Prev. 2013;14(12):7607-11. 10.7314/apjcp.2013.14.12.7607
7. Mishra S, Husain N, Awasthi NP, Pradeep Y, Roohi R, Saxena S. Liquid-based cytology: do ancillary techniques enhance detection of epithelial abnormalities? Archives of Gynecology and Obstetrics. 2018; 298:159-69.
8. Sun X, Kaufman PD. Ki-67: more than a proliferation marker. Chromosoma. 2018 Jun;127(2):175-186. doi: 10.1007/s00412-018-0659-8. Epub 2018 Jan 10.
9. Miller I, Min M, Yang C, Tian C, Gookin S, Carter D, Spencer SL. Ki67 is a Graded Rather than a Binary Marker of Proliferation versus Quiescence. Cell Rep. 2018;24(5):1105-1112.e5. 10.1016/j.celrep.2018.06.110
10. Popiel A, Piotrowska A, Sputa-Grzegrzolka P, Smolarz B, Romanowicz H, Dziegiel P, Podhorska-Okolow M, Kobierzycki C. Preliminary Study on the Expression of Testin, p16 and Ki-67 in the Cervical Intraepithelial Neoplasia. Biomedicines. 2021; 9(8):1010. https://doi.org/10.3390/biomedicines9081010
11. Singh P, Garg K, Singh A, Tripathi S. Expression of Ki-67 in premalignant and malignant lesion of cervix in tertiary care hospital in Uttar Pradesh. International Journal of Research in Medical Sciences. 2023;11(5):1575-81.
12. Zhong P, Li J, Gu Y, Liu Y, Wang A, Sun Y, Lu L. P16 and Ki-67 expression improves the diagnostic accuracy of cervical lesions but not predict persistent high risk human papillomavirus infection with CIN1. Int J Clin Exp Pathol. 2015;8(3):2979-86.
13. Takkem A, Barakat C, Zakaraia S, Zaid K, Najmeh J, Ayoub M, Seirawan MY. Ki-67 Prognostic Value in Different Histological Grades of Oral Epithelial Dysplasia and Oral Squamous Cell Carcinoma. Asian Pac J Cancer Prev. 2018;19(11):3279-3286. 10.31557/APJCP.2018.19.11.3279.
14. Ekaterina K, Irakli K, Elene K, Ana M, Mariam A. A Comparative Study of Conventional Pap Smear and Liquid-Based Cytology. Health Sci Rep. 2025;8(4): e70768. doi: 10.1002/hsr2.70768.
15. do Nascimento Santos TA, da Silva AKF, Cunha KSG, da Costa CBP, da Silva AR, Castro HC, Oliveira NSC. Current advances in cancer immunohistochemistry: a new perspective for the Ki-67 biomarker. Ecancermedicalscience. 2025; 19:1863. doi: 10.3332/ecancer.2025.1863.
16. Gahine R, Pandey V, Gajendra M, Shende PK. Analysis of Pattern of Expression of Immunohistochemical Markers p16, p53 and Ki67 on Cervical Lesions and its Correlation with Histopathology. Journal of Medical Sciences. 2025;11(1):32.doi: 10.46347/jmsh. v11.i1.24.242
17. Davey MG, Hynes SO, Kerin MJ, Miller N, Lowery AJ. Ki-67 as a Prognostic Biomarker in Invasive Breast Cancer. Cancers (Basel). 2021;13(17):4455. doi: 10.3390/cancers13174455.
18. Mitildzans A, Arechvo A, Rezeberga D, Isajevs S. Expression of p63, p53 and Ki-67 in Patients with Cervical Intraepithelial Neoplasia. Turk Patoloji Derg. 2017;33(1):9-16. 10.5146/tjpath.2016.01373.
19. Ouh YT, Kim HY, Yi KW, Lee NW, Kim HJ, Min KJ. Enhancing Cervical Cancer Screening: Review of p16/Ki-67 Dual Staining as a Promising Triage Strategy. Diagnostics (Basel). 2024;14(4):451.10.3390/diagnostics14040451.