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Evaluation of premalignant and malignant lesions in conventional pap smears by nuclear morphometry: a prospective observational study.

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Abstract

Background:
Cervical cancer remains a major preventable malignancy among women, and conventional Papanicolaou smear screening is central to early detection. However, borderline cytological interpretation between premalignant and malignant lesions is sometimes subjective.

Objectives:
To evaluate cellular and nuclear morphometric parameters in conventional Pap smears and to assess their usefulness in differentiating low-grade squamous intraepithelial lesion, high-grade squamous intraepithelial lesion, and squamous cell carcinoma.

Methods:
This prospective cross-sectional observational study was conducted in the Department of Pathology, Kamineni Institute of Medical Sciences, Narketpally, Telangana, from February 2025 to March 2026. Among 1,200 conventional Pap smears assessed during the study period, all 100 cases meeting the predefined specimen-quality criteria and having histopathological confirmation were included. Smears were classified using the Bethesda system. Digital images were analysed with ImageJ 1.44c software, and approximately 25 well-preserved, non-overlapping nuclei per case were assessed.

Results:
The age range was 21-70 years. The study included 37 normal, 23 reactive, 10 atypical squamous cells of undetermined significance, 15 low-grade squamous intraepithelial lesion (LSIL), 10 high-grade squamous intraepithelial lesion (HSIL), and 5 squamous cell carcinoma (SCC) smears. Nuclear area increased from 104.14 ± 13.89 in LSIL to 112.49 ± 21.58 in HSIL and 152.81 ± 24.58 in SCC ($p<0.001$). Nuclear perimeter was 108.97 ± 8.07, 111.01 ± 11.53, and 132.52 ± 7.34, respectively ($p<0.001$); nuclear diameter was 11.58 ± 0.69, 12.27 ± 1.10, and 14.12 ± 0.97 ($p<0.001$); and the nuclear-to-cytoplasmic ratio was 0.102 ± 0.030, 0.160 ± 0.028, and 0.222 ± 0.033 ($p<0.001$).

Conclusion:
Nuclear morphometry served as a useful objective adjunct for distinguishing premalignant and malignant cervical cytology categories.

Recommendations:
Nuclear morphometry should be incorporated as a supportive tool in borderline Pap smear interpretation, especially for differentiating low-grade, high-grade, and malignant squamous lesions.

Keywords: *Nuclear morphometry; Papanicolaou smear; Low-grade squamous intraepithelial lesion; High-grade squamous intraepithelial lesion; Squamous cell carcinoma; Bethesda system.*
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Introduction
Cervical cancer remains one of the leading causes of cancer-related morbidity and mortality among women, particularly in low- and middle-income countries. Recent global cancer estimates continue to show a substantial cervical cancer burden, despite the availability of effective prevention and screening strategies [1,2]. Persistent infection with high-risk human papillomavirus is the central biological driver of

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cervical carcinogenesis, but the progression from infection to intraepithelial lesion and invasive carcinoma usually occurs over several years [3]. This prolonged premalignant phase creates an important diagnostic window in which screening can identify precursor lesions before invasive disease develops [4].

The Papanicolaou smear has been one of the most successful cancer screening tools in clinical medicine. It is simple, economical, and suitable for large-scale cervical screening programmes, especially in settings where advanced molecular testing is not universally available [5,6]. The Bethesda system has strengthened communication between cytopathologists and clinicians by standardizing reporting categories such as atypical squamous cells of undetermined significance (ASC-US), low-grade squamous intraepithelial lesion (LSIL), high-grade squamous intraepithelial lesion (HSIL) and squamous cell carcinoma (SCC) [7,8]. These categories guide follow-up, colposcopy, biopsy and treatment decisions, making accurate cytological interpretation clinically important.

Despite its public health value, conventional cytology has important interpretive limitations. Reactive atypia, inflammation, hormonal changes, atrophy and sampling artefacts can mimic epithelial abnormalities. In addition, reproducibility is lower in equivocal and borderline categories, and interobserver variation is well documented in cervical cytology and histology [9,10]. The distinction between LSIL, HSIL and early invasive squamous carcinoma is usually based on nuclear enlargement, hyperchromasia, chromatin irregularity, nuclear membrane changes, cellular maturation and the nuclear-to-cytoplasmic ratio. These features are diagnostically meaningful, but routine interpretation remains partly subjective.

Computer-assisted morphometry helps convert visual impressions into measurable variables. Parameters such as nuclear area, nuclear perimeter, nuclear diameter, cellular area, and the nuclear-to-cytoplasmic ratio can objectively represent the architectural and nuclear changes associated with neoplastic transformation [11,12]. Previous studies on cervicovaginal smears have shown that nuclear morphometry can differentiate premalignant from malignant lesions and can add value in difficult cytological categories [13,14]. The present study was therefore conducted to evaluate nuclear and cellular morphometric parameters in conventional Pap smears. The objectives were to assess the distribution of cytological categories by age, compare morphometric parameters among LSIL, HSIL and SCC, and determine the usefulness of nuclear morphometry as an adjunctive objective tool in distinguishing premalignant and malignant cervical smears in routine diagnostic practice.

Methodology

Study design and setting

A prospective cross-sectional observational design was used. The study was conducted in the Department of Pathology, Kamineni Institute of Medical Sciences (KIMS), Sreepuram, Narketpally, Nalgonda district, Telangana, India, from February 2025 to March 2026. KIMS is a tertiary-care teaching institution in a rural setting, approximately 89 km from Hyderabad, with an attached teaching hospital of about 1,060 beds and broad- and super-specialty services. Clinical services include general medicine, general surgery, paediatrics, obstetrics and gynaecology, orthopaedics, ophthalmology, otorhinolaryngology, emergency medicine, pathology, microbiology, biochemistry, radiodiagnosis and blood-bank services. The hospital receives patients from Narketpally and surrounding communities of Nalgonda district, providing a suitable tertiary-care setting for cervical cytology and histopathological correlation.

Participants and sampling

The sampling frame comprised all 1,200 conventional cervical Pap smears received in the pathology department during the study period. Case selection used consecutive total enumeration of eligible specimens rather than random sampling: every smear that satisfied the predefined eligibility criteria and had corresponding histopathological confirmation was considered. Exactly 100 cases met these requirements, and all were included. The study population comprised women aged 21-70 years with smears classified as normal, reactive, atypical squamous cells of undetermined significance (ASC-US), LSIL, HSIL or SCC.

Study size

Because the study used total enumeration of eligible, histopathologically confirmed cases, the achieved sample was determined by the number of qualifying specimens during the study period. To demonstrate adequacy of the achieved sample, a conservative single-proportion precision calculation was applied: $n = Z^2p(1-p)/d^2$, where $Z=1.96$ for a 95% confidence level, $p=0.50$ (used when no stable local prevalence estimate is available, yielding the maximum sample requirement), and $d=0.10$ absolute precision. Thus, $n=(1.96)^2\times0.50\times0.50/(0.10)^2=96.04$, giving a minimum of 97 cases. The final inclusion of 100 eligible cases therefore exceeded this minimum.

Inclusion criteria

Conventional cervical Pap smears reported as normal, reactive, or squamous epithelial abnormalities and supported by histopathological confirmation were included. Squamous abnormalities selected for morphometric comparison included LSIL, HSIL, and SCC. Cases with

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adequate cellularity, preserved nuclear morphology and satisfactory image quality were considered eligible.

Exclusion criteria

Pap smears without histopathological confirmation, inadequate smears, poorly preserved slides, obscuring haemorrhage or inflammation, broken slides, and cases without measurable representative nuclei were excluded. Glandular epithelial abnormalities were not included because the study focused on squamous lesions.

Study variables and diagnostic criteria

The principal quantitative outcome variables were nuclear area, nuclear perimeter, nuclear diameter, and nuclear-to-cytoplasmic (N: C) ratio. Secondary morphometric variables were cellular area and cellular perimeter. The principal categorical explanatory variable was cytological diagnosis (normal, reactive, ASC-US, LSIL, HSIL or SCC), and age was recorded as a descriptive demographic variable. Cervical cytology was classified according to the Bethesda system. LSIL was identified by squamous cells showing koilocytic change, nuclear enlargement, hyperchromasia and mild nuclear membrane irregularity; HSIL was characterized by smaller less-mature cells with scant cytoplasm, a high N: C ratio, hyperchromatic nuclei, coarse chromatin and more pronounced nuclear contour irregularity; SCC was diagnosed when overtly malignant squamous cells showed marked pleomorphism, irregular hyperchromatic chromatin and other cytological features of malignant transformation, with corresponding histopathological confirmation used as the reference diagnosis.

Data sources and measurement

Data were obtained from the departmental cervical cytology records, the corresponding Pap-smear slides, histopathology reports, and the digital morphometry measurements generated for the study. Slides were reviewed by light microscopy, and representative fields were selected at 40× objective magnification. Digital images of 600×400 pixels were captured under a uniform imaging protocol and analysed using ImageJ 1.44c. Approximately 25 well-preserved, non-overlapping representative nuclei were measured in each case. Cellular area and perimeter described the measured size and boundary of the selected cell; nuclear area, perimeter and diameter quantified nuclear size and contour; and the N: C ratio represented the relative nuclear component compared with the cytoplasmic component for the selected cells. Case-level values were derived from the measured cells and entered into the analysis dataset.

Quantitative variables and statistical analysis

Morphometric measurements were retained as continuous quantitative variables and summarized as mean ± standard deviation; no arbitrary cut-off values were created for nuclear area, nuclear perimeter, nuclear diameter, cellular area, cellular perimeter or N: C ratio. Age was grouped into 10-year bands only for descriptive presentation in Table 1 to show the distribution of cytological categories across clinically interpretable age ranges. Diagnostic groups (LSIL, HSIL and SCC) were defined by Bethesda categories rather than data-driven thresholds. Student's t-test was used for prespecified pairwise comparisons, while one-way analysis of variance was used when comparing quantitative morphometric measurements across more than two diagnostic groups. A two-sided p-value <0.05 was considered statistically significant.

Bias control and ethical considerations

Selection bias was reduced by applying the same predefined eligibility criteria to all Pap smears received during the study period and by including all eligible histopathologically confirmed cases. Measurement bias was minimized through a uniform image-acquisition protocol, software-based morphometry, selection of well-preserved non-overlapping cells, and exclusion of slides in which representative nuclei could not be measured reliably. Ethical approval was obtained from the Institutional Ethics Committee, Kamineni Institute of Medical Sciences, Narketpally, Nalgonda, Telangana, India. Patient identifiers were not used in the analytical dataset, and confidentiality was maintained throughout the study.

Results

Participant flow: A total of 1,200 conventional Pap smears were assessed during the study period. After application of the eligibility criteria, 1,100 cases were excluded because they lacked histopathological confirmation and/or had inadequate or poorly preserved smear material, obscuring haemorrhage or inflammation, broken slides, glandular abnormalities outside the study scope, or no measurable representative nuclei. The remaining 100 cases fulfilled all criteria, underwent morphometric assessment, and were included in the final analysis; there was no loss after inclusion. The age of the included women ranged from 21 to 70 years, and the final cytological distribution was 37 normal, 23 reactive, 10 ASC-US, 15 LSIL, 10 HSIL, and 5 SCC cases.

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Participant Flow Diagram

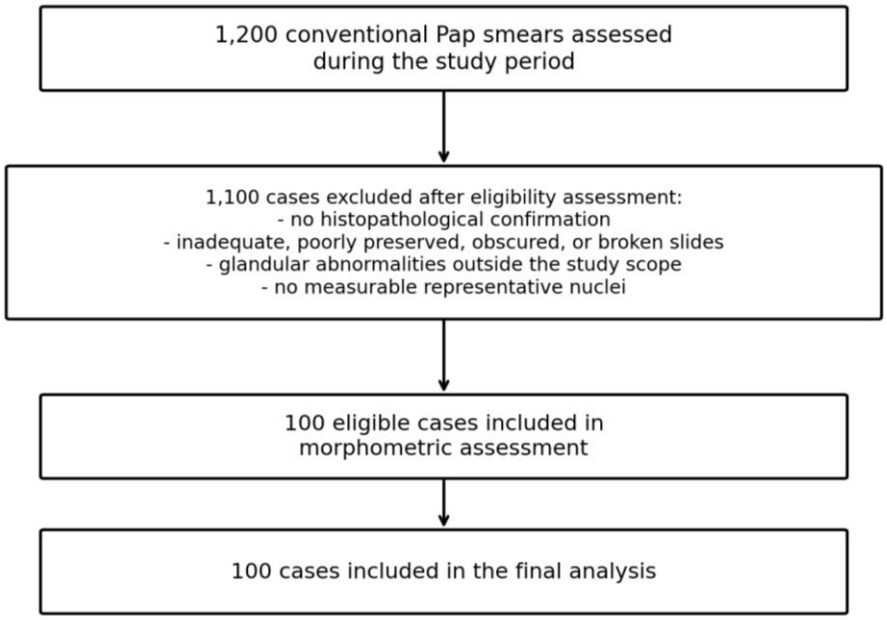

Table 1. Age-wise distribution of cytological categories

Age group (years)	Normal (n=37)	Reactive (n=23)	ASC-US (n=10)	LSIL (n=15)	HSIL (n=10)	SCC (n=5)	Total n (%)
20-30	21	10	0	4	0	0	35 (35.0)
31-40	10	8	3	2	2	0	25 (25.0)
41-50	6	5	6	2	1	1	21 (21.0)
51-60	0	0	1	4	4	1	10 (10.0)
61-70	0	0	0	3	3	3	9 (9.0)
Total	**37**	**23**	**10**	**15**	**10**	**5**	**100 (100.0)**

Normal and reactive smears were observed predominantly in the 20-30-year and 31-40-year age groups. LSIL was distributed across a wider age range, with cases seen in younger as well as older women. HSIL cases were more frequent from 51 to 70 years. SCC was concentrated in the older age groups, with the highest frequency in the 61-70-year age group. This pattern indicates increasing representation of high-grade lesions and carcinoma with advancing age.

Cytological assessment showed that LSIL smears had squamous cells arranged singly and in sheets, with enlarged hyperchromatic nuclei, mild nuclear membrane irregularity, and increased nuclear-to-cytoplasmic ratio. HSIL smears showed smaller cells with reduced cytoplasm, higher nuclear-to-cytoplasmic ratio, hyperchromatic nuclei, prominent nuclear irregularity, and coarse chromatin. SCC smears demonstrated malignant squamous cells in scattered and syncytial-like groups, marked nuclear pleomorphism, irregular chromatin, macronucleoli and features consistent with invasive malignant transformation.

Morphometric analysis of LSIL, HSIL and SCC showed that nuclear parameters were more discriminatory than cellular parameters. Nuclear area increased from 104.14 ± 13.89 in LSIL to 112.49 ± 21.58 in HSIL and 152.81 ± 24.58 in SCC. Nuclear perimeter, nuclear diameter, and nuclear-to-cytoplasmic ratio were also highest in SCC. The comparative morphometric parameters are shown in Table 2.

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Table 2. Nuclear and cellular morphometric parameters by cytological group

Parameter	LSIL Mean ± SD	HSIL Mean ± SD	SCC Mean ± SD	t value	p value
Cell area	1084.20 ± 298.81	698.60 ± 199.94	674.09 ± 93.21	0.826	0.427
Cell perimeter	483.09 ± 390.41	311.97 ± 21.84	336.15 ± 36.07	-4.613	0.001
Nuclear area	104.14 ± 13.89	112.49 ± 21.58	152.81 ± 24.58	-4.613	<0.001
Nuclear perimeter	108.97 ± 8.07	111.01 ± 11.53	132.52 ± 7.34	-5.285	<0.001
Nuclear diameter	11.58 ± 0.69	12.27 ± 1.10	14.12 ± 0.97	-5.551	<0.001
Nuclear-to-cytoplasmic ratio	0.102 ± 0.030	0.160 ± 0.028	0.222 ± 0.033	-6.766	<0.001

Cell area did not show a statistically significant difference (p=0.427). In contrast, nuclear area, nuclear perimeter, nuclear diameter, and nuclear-to-cytoplasmic ratio each showed statistically significant differences (all p<0.001) and demonstrated a consistent increase toward SCC. These findings suggest that nuclear parameters provide stronger objective discrimination between premalignant and malignant squamous lesions than cellular size alone. Representative cytology images and ImageJ-based analysis are shown in Figures 1-4.

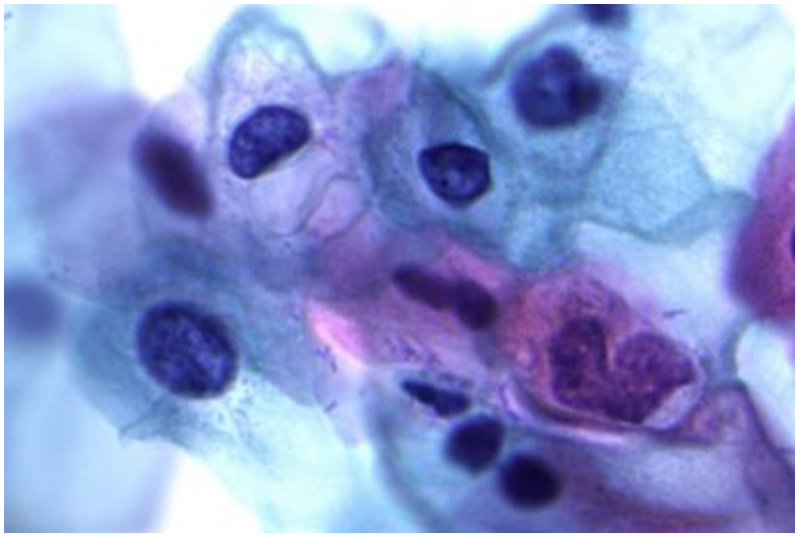

Figure 1. Low-grade squamous intraepithelial lesion on conventional Pap smear.

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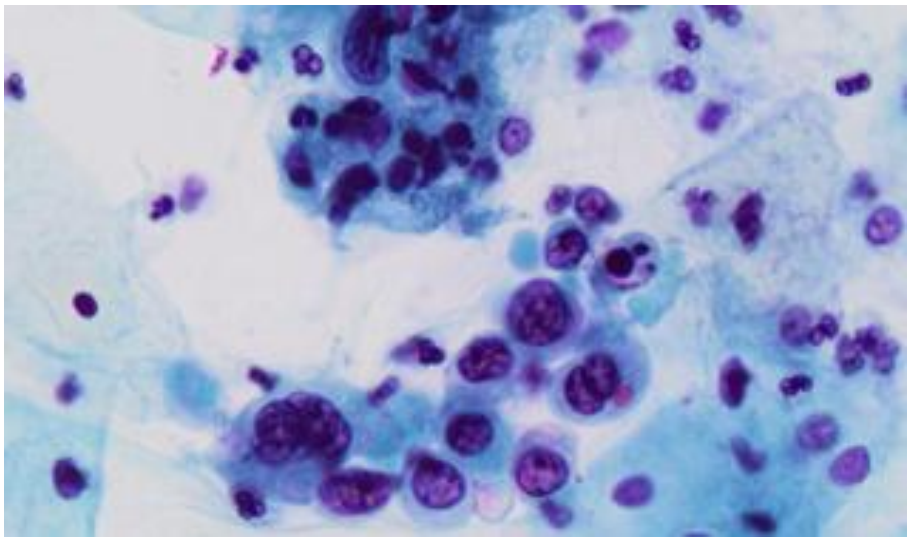

Figure 2. High-grade squamous intraepithelial lesion on conventional Pap smear.

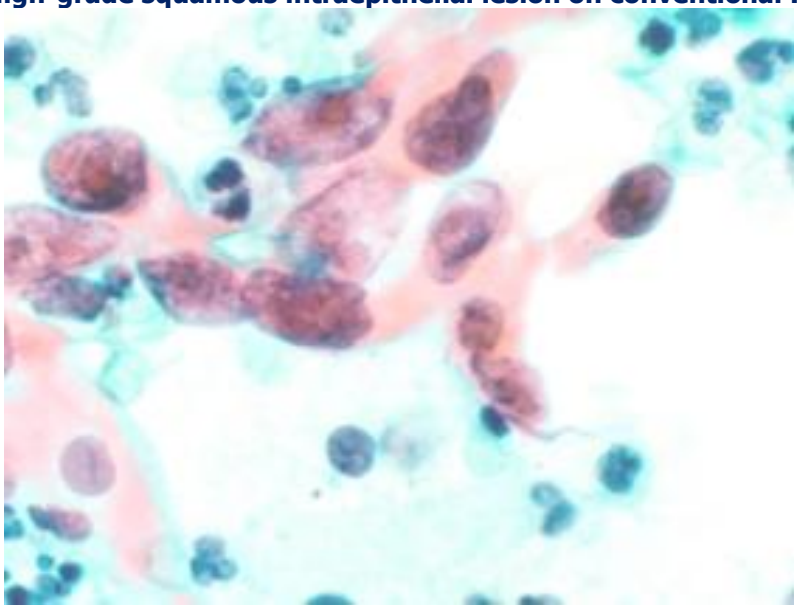

Figure 3. Squamous cell carcinoma on conventional Pap smear.

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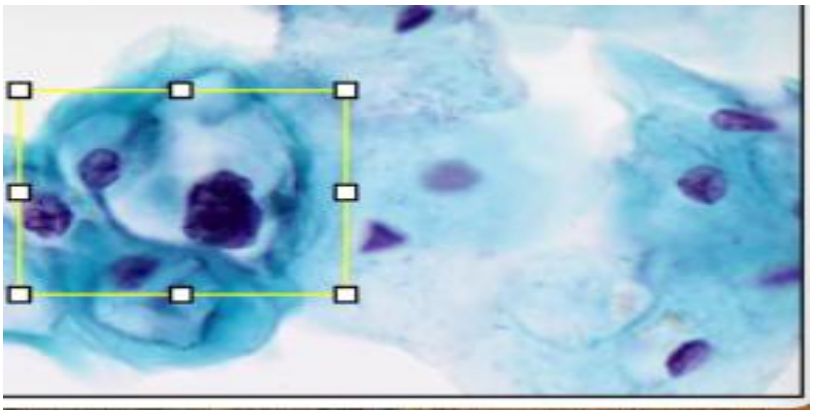

Figure 4. Morphometric analysis of a Pap smear image.

Discussion

The present study demonstrates that quantitative nuclear morphometry can provide objective support for the cytological distinction of premalignant and malignant squamous lesions. Among 100 analysed cases, 15 were LSIL, 10 were HSIL, and 5 were SCC. Higher-grade lesions were concentrated in older women: 7 of 10 HSIL cases (70.0%) and 4 of 5 SCC cases (80.0%) occurred between 51 and 70 years. This age shift is consistent with the long natural history of persistent high-risk human papillomavirus infection, progressive intraepithelial neoplasia, and eventual invasive transformation in a subset of untreated lesions [3,4].

The strongest finding was the stepwise increase in nuclear measurements with increasing lesion severity. Mean nuclear area rose from 104.14 ± 13.89 in LSIL to 112.49 ± 21.58 in HSIL and 152.81 ± 24.58 in SCC ($p<0.001$). Nuclear perimeter similarly increased from 108.97 ± 8.07 to 111.01 ± 11.53 and 132.52 ± 7.34 ($p<0.001$), while nuclear diameter increased from 11.58 ± 0.69 to 12.27 ± 1.10 and 14.12 ± 0.97 ($p<0.001$). The N: C ratio showed the clearest graded pattern, rising from 0.102 ± 0.030 in LSIL to 0.160 ± 0.028 in HSIL and 0.222 ± 0.033 in SCC ($p<0.001$). These data indicate progressive nuclear enlargement and increasing nuclear dominance as cytological atypia advances toward invasive carcinoma.

These findings are concordant with earlier nuclear-morphometry studies. Divya Rani et al. reported that nuclear area, perimeter, and diameter were statistically useful in separating premalignant from malignant cervicovaginal smears and concluded that nuclear morphometry provides an objective adjunct to routine Pap-smear interpretation [13]. Mishra et al. likewise demonstrated that cytonucleomorphometric parameters differed across squamous-cell abnormalities and could assist discrimination of abnormal cervical cytology categories [14]. The direction of change in the present series - progressively larger nuclei and a higher N: C ratio from LSIL through HSIL to SCC - therefore supports the biological and diagnostic consistency of these measurable features.

In contrast to the nuclear variables, cellular area was less informative and did not show a statistically significant group difference ($p=0.427$). This may reflect the greater susceptibility of cytoplasmic size to cell maturation, sampling, fixation, spreading and preservation. Nuclear morphology is more directly related to dysplastic transformation, including increased DNA content, hyperchromasia, contour irregularity and loss of normal maturation. The significant change in cellular perimeter ($p=0.001$), together with the much stronger and more consistent significance of nuclear area, perimeter, diameter and N: C ratio, suggests that nuclear measures are more robust candidate parameters for objective diagnostic support.

From a practical perspective, ImageJ-based measurement of approximately 25 representative nuclei per case is feasible in a routine pathology laboratory and may be especially helpful when conventional microscopy yields borderline interpretation. Interobserver variability is recognized in cervical cytology, particularly around equivocal and low- versus high-grade categories [9,10]. Morphometry cannot replace expert cytological assessment or histopathological confirmation, but it can add reproducible quantitative evidence to the diagnostic process. Standardized image capture, calibration procedures, cell-selection rules and laboratory-specific quality control would be necessary before wider implementation, while larger multicentre studies should define validated diagnostic thresholds and assess performance against HPV testing and histopathology.

Generalizability

The findings apply to tertiary care pathology settings where conventional Pap smears, histopathological correlation and digital microscopy facilities are available. Because the study was single-centre and used a limited number of abnormal smears, direct extrapolation to community screening programmes requires caution. Still, the observed

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morphometric trend across LSIL, HSIL and SCC is biologically coherent and supports further validation through multicentre studies with standardized imaging protocols and clearly defined measurement thresholds for routine reporting.

Conclusion

This prospective observational study demonstrated that nuclear morphometry is a valuable objective adjunct in cervical cytology. Nuclear area, nuclear perimeter, nuclear diameter, and nuclear-to-cytoplasmic ratio increased progressively from LSIL to HSIL and SCC, with the highest values observed in squamous cell carcinoma. Cellular area was less useful for separating HSIL from SCC. These findings indicate that measurable nuclear parameters can support routine Pap smear interpretation, especially in diagnostic gray zones between premalignant and malignant lesions. Combining clinical assessment, conventional cytomorphology, histopathological correlation and morphometric data can improve diagnostic confidence and guide timely management of women with abnormal cervical smears in tertiary care settings with better reproducibility in daily clinical practice.

Limitations

This single-centre study had a limited number of LSIL, HSIL and SCC cases. HPV DNA correlation was not performed for all Pap smears. Glandular epithelial abnormalities were outside the study scope. Reference cut-off values for morphometric parameters were unavailable, limiting threshold-based interpretation. Manual selection of representative nuclei introduced observer-dependent measurement influence despite software-based analysis, and follow-up outcome data were not assessed after diagnosis.

Recommendations

Nuclear morphometry should be used as an adjunctive tool in routine cervical cytology, particularly for borderline cases where LSIL, HSIL and SCC are difficult to separate on morphology alone. Pathology departments can adopt standardized image acquisition, calibration and nuclear selection protocols to improve reproducibility. Future research should include larger multicentre cohorts, HPV DNA correlation, histopathological grading and receiver operating characteristic analysis to identify diagnostic cut-off values for nuclear area, perimeter, diameter and nuclear-to-cytoplasmic ratio. Training cytotechnologists and pathologists in digital morphometry will support consistent use in routine screening laboratories and strengthen quality assurance in cervical cancer prevention programmes.

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Abbreviations

ASC-US: Atypical squamous cells of undetermined significance
CA: Cellular area
CIN: Cervical intraepithelial neoplasia
HPV: Human papillomavirus
HSIL: High-grade squamous intraepithelial lesion
LSIL: Low-grade squamous intraepithelial lesion
N: C ratio: Nuclear-to-cytoplasmic ratio
NA: Nuclear area
Pap: Papanicolaou
SCC: Squamous cell carcinoma

Source of funding

The study received no external funding.

Conflict of interest

The authors declare no conflict of interest.

Author Contributions

Dr. Y. Divyaprafulla contributed to the conception and design of the study, interpretation of results, review of literature, preparation of the first draft of the manuscript, statistical analysis, data interpretation, and critical revision of the manuscript. **Dr. Mounika Reddy Guntaka** contributed to the conception and design of the study, review of literature, and interpretation of results. **Dr. Himabindu G** contributed to the review of literature, interpretation of results, and critical review of the manuscript.

Data Availability

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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