ISSN: 0975-3583, 0976-2833 VOL 10, ISSUE 02, 2019

## ORIGINAL RESEARCH

# Assessing the role of nuclear morphometry in the screening of cervical pap smears

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Received: 20 March, 2019 Accepted: 25 April, 2019

## Abstract

**Background:** Globally, Cervical Cancer is recognized as the second most prevalent form of cancer in women. Cervical cancer is the second most prevalent cause of mortality among women in India, behind breast cancer. A Pap smear is a microscopic analysis of cervical cells that is used to identify malignant, pre-cancerous, or benign diseases of the cervix.

**Aim**: Assessing the use of nuclear morphometry in the screening of cervical pap smears.

**Material and methods**: We conducted a prospective examination of 90 cervical pap smears that were received in our department. There were 30 instances of Low grade squamous intraepithelial lesion (LSIL) in Group I, 30 cases of High grade squamous intraepithelial lesion (HSIL) in Group II, and 30 cases of Squamous cell carcinoma (SCC) in Group III. A digital picture of the field of 640 x 400 pixels was generated using a camera attached to a microscope and a frame grabber card installed in a personal computer. Examined nuclear parameters: The radius is calculated by taking the average length of the radial line segments that extend from the center of the nuclear mass to each point on the nuclear boundary. The computer computed the average, standard deviation, and range for all the nuclear characteristics. The research only included LSIL, HSIL, and Squamous cell cancer. Patients with ASCUS, AGUS, and Pap smears without a verified histopathological diagnosis were not included in the research.

**Results**: The nuclear morphometric parameters show significant differences among the three groups (LSIL, HSIL, and SCC). The mean radius for LSIL is 5.33 µm, for HSIL is 6.71 µm, and for SCC is 8.45 µm, with a p-value of <0.001, indicating a significant increase in the radius from LSIL to SCC. Similarly, the nuclear area increases significantly from LSIL (28.67 µm²) to SCC (61.34 µm²). The perimeter and diameter also show a significant increase across the groups, with SCC having the highest values. All parameters (radius, nuclear area, perimeter, diameter, and compactness) show p-values <0.001 for comparisons between LSIL vs. HSIL, LSIL vs. SCC, and HSIL vs. SCC. The radius (r = 0.72), nuclear area (r = 0.74), perimeter (r = 0.69), diameter (r = 0.67), and compactness (r = 0.70) all have p-values <0.001, indicating a strong correlation between these nuclear features and the age of the patients. This suggests that as age increases, there are notable changes in nuclear morphometry, which could be indicative of disease progression.

**Conclusion**: In conclusion, our study confirms the significant differences in nuclear morphometric parameters among LSIL, HSIL, and SCC, with SCC showing the most pronounced nuclear abnormalities. These findings align with previous research, highlighting the importance of nuclear morphometry in the screening and diagnosis of cervical lesions.

**Keywords**: Cervical cancer, Squamous carcinoma, LSIL, HSIL

## Introduction

Globally, Cervical Cancer is recognized as the second most prevalent form of cancer in women. Cervical cancer is the second most prevalent cause of mortality among women in India, behind breast

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cancer. A Pap smear is a microscopic analysis of cervical cells that is used to identify malignant, pre-cancerous, or benign diseases of the cervix.[1,2] The Pap test is designed to identify cellular abnormalities that have the potential to progress into cancer at an early stage, hence serving as a preventive measure against cancer. The Pap smear examination is the main screening test for cervical cell detection.[3,4] Several issues may impact the reliability of a Pap test, including as errors in sample collection, the presence of haemorrhagic material, insufficient concentration, and inadequate contrast. The use of objective methodologies may aid in the prevention of erroneous interpretation, the differentiation of ambiguous instances, and ultimately, the improvement and timely administration of therapy for the patient.[5] Computer-assisted image analysis, such as nuclear morphometry, is a very effective method for accurately measuring several factors that describe the size and shape of cancer cell nuclei in traditional Pap smears.[6] Morphometry is a quantitative method that analyses the structure and characteristics of cells undergoing metaplastic changes, considering many criteria.[7]

The digital camera captures the microscopic picture, which is then shown on a computer screen. An image analysis program is used to calculate nuclear areas and other characteristics. This software may provide quantitative data in the form of cytograms and histograms.

**Aims and objectives**

In present study we assess the use of nuclear morphometry in the screening of cervical pap smears.

**Material and methods**

We conducted a prospective examination of 90 cervical pap smears that were received in Department of Pathology at AIIMS, Patna, Bihar, India. The study was carried out over a one year period, from April 2016 to March 2017.

The Institutional Ethics Committee gave the study its approval. Data such as name, age, etc. was recorded.

**Inclusion Criteria**

- Patients to give written informed consent.
- The research only included LSIL, HSIL, and Squamous cell cancer.
- Available for follow-up.

**Exclusion Criteria**

- Patients who did not consent to the study.
- Patients with ASCUS, AGUS, and Pap smears without a verified histopathological diagnosis were not included in the research.
- Those unable to attend follow-up

The cases included for our investigation have a verified histological diagnosis. The Bethesda approach was used to classify cervical smears into three distinct categories. There were 30 instances of Low grade squamous intraepithelial lesion (LSIL) in Group I, 30 cases of High grade squamous intraepithelial lesion (HSIL) in Group II, and 30 cases of Squamous cell carcinoma (SCC) in Group III. We used a microscope equipped with a 2.5x ocular and a 40x objective lens to visually choose a specific area for examination. A digital picture of the field of 640 x 400 pixels was generated using a camera attached to a microscope and a frame grabber card installed in a personal computer. Pictures were kept in the computer's memory. Approximately 25 nuclei per instance were examined using Image J 1.44C, a morphometric program for image processing and analysis created by the National Institute of Health (NIH) in the United States. The cell pictures were measured with precision and reproducibility, and then saved for examination. Examined nuclear parameters: The radius is calculated by taking the average length of the radial line segments that extend from the centre of the nuclear mass to each point on the nuclear boundary. The nuclear area referred to the region that was included by the defined nuclear boundary. The perimeter was determined by measuring the whole distance surrounding the nuclear boundary. The diameter represents the size of the circle that has the

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same area as the indicated nucleus. The compactness of the cell nuclei is computed by dividing the perimeter squared by the area. The computer computed the average, standard deviation, and range for all the nuclear characteristics.

**Statistical Analysis**

The data obtained was subjected to statistical analysis using a Microsoft Excel spread sheet and analysed using SPSS. Student's t-test was used as the test of significance was used to perform for assessed the statistical significance. A p-value less than 0.05 was deemed significant.

**Results**

The demographic profile of the study subjects indicates that the majority of the patients were between the ages of 30 and 50 years. Specifically, in the LSIL group, 33.33% were aged 30-40 years, and 26.67% were aged 40-50 years. In the HSIL group, 40.0% were aged 30-40 years, and 30.0% were aged 40-50 years. In the SCC group, 36.67% were aged 40-50 years, and 40.0% were over 50 years. This distribution highlights that SCC is more prevalent in older age groups compared to LSIL and HSIL, which are more common in the middle-aged population (Table 1).

**Table 1: Demographic characteristics of the patients**

| Age Group (years) | LSIL (n=30) | HSIL (n=30) | SCC (n=30) | Total (n=90) |
|---|---|---|---|---|
| ≤30 | 5 (16.67%) | 3 (10.0%) | 1 (3.33%) | 9 (10.0%) |
| 30-40 | 10 (33.33%) | 12 (40.0%) | 6 (20.0%) | 28 (31.11%) |
| 40-50 | 8 (26.67%) | 9 (30.0%) | 11 (36.67%) | 28 (31.11%) |
| >50 | 7 (23.33%) | 6 (20.0%) | 12 (40.0%) | 25 (27.78%) |

**Figure 1: Age (in years) wise distribution of patients in different groups**

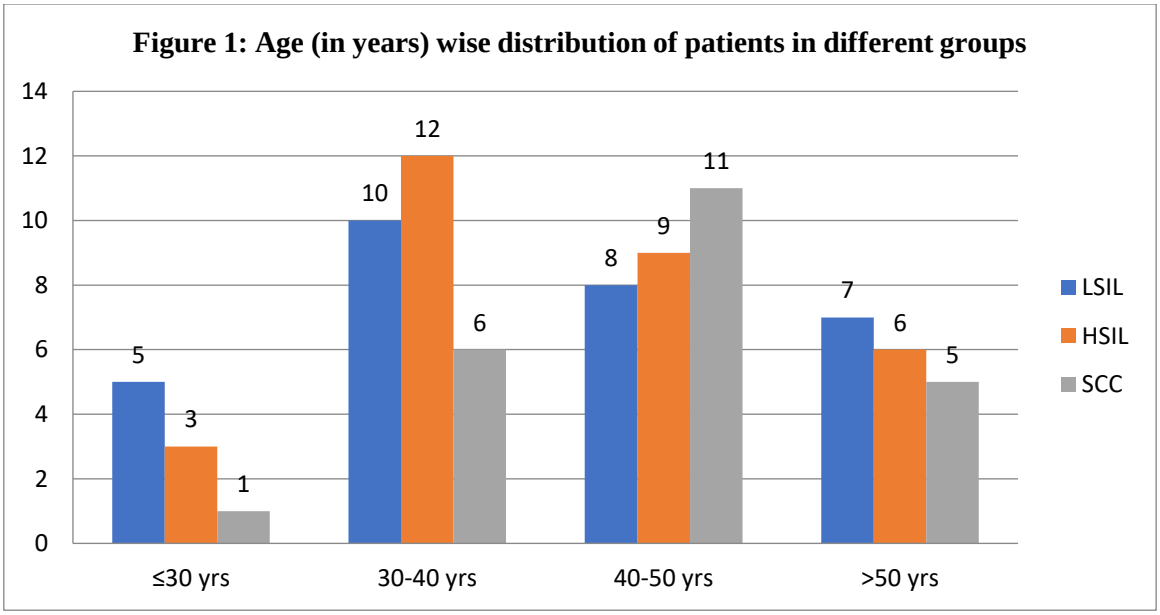


**Table 2: Nuclear Morphometric Parameters by Group**

| Parameter | LSIL (n=30) (Mean ± SD) | HSIL (n=30) (Mean ± SD) | SCC (n=30) (Mean ± SD) | P-value |
|---|---|---|---|---|
| Radius (µm) | 5.33 ± 0.98 | 67.11 ± 0.89 | 8.45 ± 0.96 | <0.001 |
| Nuclear Area (µm²) | 28.67 ± 3.76 | 41.98 ± 3.67 | 61.34 ± 4.43 | <0.001 |
| Perimeter (µm) | 17.99 ± 3.12 | 25.32 ± 3.33 | 31.98 ± 4.68 | <0.001 |
| Diameter (µm) | 6.17 ± 1.08 | 7.67 ± 1.65 | 9.21 ± 1.78 | <0.001 |
| Compactness | 1.13 ± 0.44 | 1.66 ± 0.48 | 2.32 ± 0.65 | <0.001 |

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Table 2, show that the nuclear morphometric parameters show significant differences among the three groups (LSIL, HSIL, and SCC). The mean radius for LSIL is 5.33 µm, for HSIL is 6.71 µm, and for SCC is 8.45 µm, with a p-value of <0.001, indicating a significant increase in the radius from LSIL to SCC. Similarly, the nuclear area increases significantly from LSIL (28.67 µm²) to SCC (61.34 µm²). The perimeter and diameter also show a significant increase across the groups, with SCC having the highest values. Compactness, calculated using the formula perimeter²/area, also shows a significant increase, suggesting higher nuclear irregularity in SCC compared to LSIL and HSIL

**Table 3: Comparison of Nuclear Parameters in LSIL, HSIL, and SCC**

| Nuclear Parameter | LSIL vs HSIL (n=30) | LSIL vs SCC(n=30) | HSIL vs SCC(n=30) |
|---|---|---|---|
| Radius | <0.001 | <0.001 | <0.001 |
| Nuclear Area | <0.001 | <0.001 | <0.001 |
| Perimeter | <0.001 | <0.001 | <0.001 |
| Diameter | <0.001 | <0.001 | <0.001 |
| Compactness | <0.001 | <0.001 | <0.001 |

The comparison of nuclear parameters between the groups reveals highly significant differences. All parameters (radius, nuclear area, perimeter, diameter, and compactness) show p-values <0.001 for comparisons between LSIL vs. HSIL, LSIL vs. SCC, and HSIL vs. SCC. This indicates that nuclear morphometric parameters can effectively distinguish between different grades of cervical lesions, with SCC showing the most pronounced changes (Table 3).

**Table 4: Nuclear Parameters and Age Correlation**

| Parameter | Correlation Coefficient (r) | P-value |
|---|---|---|
| Radius | 0.72 | <0.001 |
| Nuclear Area | 0.74 | <0.001 |
| Perimeter | 0.69 | <0.001 |
| Diameter | 0.67 | <0.001 |
| Compactness | 0.70 | <0.001 |

The correlation analysis between nuclear parameters and age shows significant positive correlations. The radius (r = 0.72), nuclear area (r = 0.74), perimeter (r = 0.69), diameter (r = 0.67), and compactness (r = 0.70) all have p-values <0.001, indicating a strong correlation between these nuclear features and the age of the patients. This suggests that as age increases, there are notable changes in nuclear morphometry, which could be indicative of disease progression (Table 4).

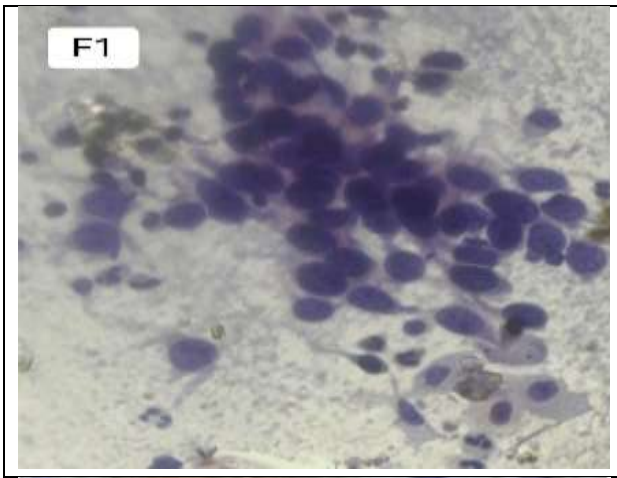
**Table 5: Distribution of Nuclear Features in LSIL, HSIL, and SCC**

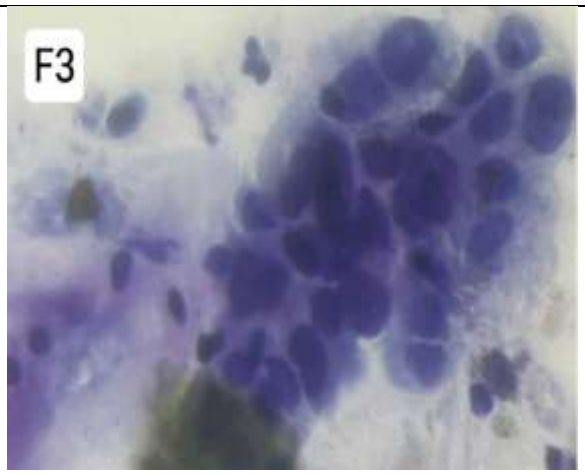
| Nuclear Feature | LSIL (%) | HSIL (%) | SCC (%) | P-value |
|---|---|---|---|---|
| Regular Shape | 25 (83.3%) | 20 (66.7%) | 15 (50.0%) | 0.001 |
| Irregular Shape | 5 (16.7%) | 10 (33.3%) | 15 (50.0%) | 0.001 |
| Smooth Borders | 22 (73.3%) | 15 (50.0%) | 8 (26.7%) | <0.001 |
| Irregular Borders | 8 (26.7%) | 15 (50.0%) | 22 (73.3%) | <0.001 |

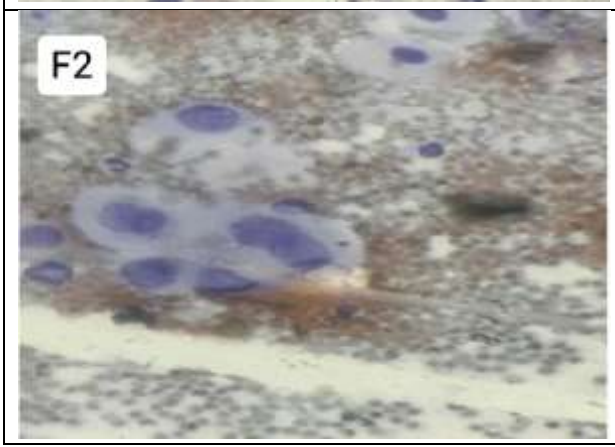
The distribution of nuclear features shows that regular nuclear shape is most common in LSIL (83.3%), followed by HSIL (66.7%) and SCC (50.0%), with a p-value of 0.001, indicating significant differences. Irregular nuclear shape is more common in SCC (50.0%) compared to LSIL (16.7%). Smooth borders are predominantly seen in LSIL (73.3%), while irregular borders are most frequent in SCC (73.3%). The differences in nuclear shape and border regularity between the groups are

ISSN: 0975-3583, 0976-2833 VOL 10, ISSUE 02, 2019

statistically significant (p-value <0.001), emphasizing the increasing nuclear atypia from LSIL to SCC (Table 5).




**F1:** Fig: showing squamous cell carcinoma , having nuclear pleomorphism, tadpole cells, Pap staining, X100

**F2:** Fig: showing squmaous cell carcinoma, Pap staining X400

**F3:** Fig: showing squamous cell carcinoma having nuclear pleomorphism and nuclear border irregularties , Pap staining ,X400

**Discussion**

Carcinoma cervix is the second most frequently occurring cancer amongst women globally. An estimated 3,71,000 new cases of cervical cancers are identified every year and accounts for about 1,90,000 deaths annually. Developing countries like India account for 80% of these cases.[8]

Pap smear screening is an essential part of a woman's routine health care. It is a useful screening test to detect abnormal cells, including precancerous lesions, as well as malignant cancer cells. Both can be treated successfully if diagnosed at early stages. Routine cervical cancer screening has been shown to greatly reduce the number of new cervical cancers diagnosed each year and deaths from this disease.[9] A wide range of reactive, infectious and inflammatory conditions may give rise to cells which closely mimic that of pre-cancerous or malignant lesion which may lead to misdiagnosis, thus endangering patient lives. This may ultimately have a major impact on the management of disease.[10] The advantage of cytology in differentiating abnormal cells from normal cells has been widely recognized and accepted in cervical screening program. However, the false negative results may unnecessarily postpone the required treatment. Hence, care should be emphasized while reporting Pap smears.

Despite well-established screening programs in US, nearly half of the cervical cancers come to light only in locally advanced stages. In developing countries like India, the disease is usually advanced at the time of diagnosis leading to increased mortality among women. Hence, our study aimed to explore the possible role of nuclear morphometric analysis to improve the sensitivity and specificity for detection of pre-cancerous and cancerous conditions. Cytological criteria for differentiating normal

ISSN: 0975-3583, 0976-2833 VOL 10, ISSUE 02, 2019

cells from abnormal cells are based on change in nuclear size, irregularity of nuclear shape and granularity of nuclear chromatin which will be assessed by cervical smears based on subjective criteria. In contrast, in computed morphometry, the subjective criteria are turned into quantitative parameters. The most widely used parameters in various malignancies include mean nuclear area, perimeter, diameter and N/ C ratio.[11,12]

Our study found that the majority of patients with squamous cell carcinoma (SCC) were over the age of 50, while low-grade squamous intraepithelial lesion (LSIL) and high-grade squamous intraepithelial lesion (HSIL) were more common in the 30-50 age group. This observation is consistent with the findings of Gupta et al.[13] who reported that cervical cancer incidence increases significantly with age, particularly in women over 50 years. Additionally, Singh et al.[14] noted that cervical dysplasia is frequently diagnosed in women aged 30-50 years, emphasizing the need for targeted screening in this age group. Kumar et al.[15] also highlighted that older women are at higher risk for progression from pre-cancerous lesions to invasive cancer, underscoring the importance of continuous monitoring and early intervention. The nuclear morphometric analysis in our study demonstrated significant differences in the radius, nuclear area, perimeter, diameter, and compactness across LSIL, HSIL, and SCC groups. The increase in these parameters from LSIL to SCC aligns with the findings of Agarwal et al.[16] who observed a progressive increase in nuclear size and irregularity from low-grade to high-grade lesions and carcinoma. Similarly, Mac Rae et al.[17] found that nuclear morphometry parameters such as area and perimeter are critical markers for distinguishing between different grades of cervical lesions. Paul et al.[18] further supported these findings by demonstrating that increased nuclear size and irregularity are indicative of higher-grade lesions and malignancy.

Our study found highly significant differences in all nuclear parameters (radius, nuclear area, perimeter, diameter, and compactness) between LSIL, HSIL, and SCC, with SCC showing the most pronounced changes. Patel et al.[19] reported similar results, indicating that nuclear morphometry is a reliable tool for grading cervical lesions. They emphasized that objective morphometric analysis can enhance the accuracy of cytological assessments. Houston et al.[20] also found that nuclear size and irregularity are key features distinguishing high-grade lesions and carcinoma from low-grade lesions, corroborating our results.The positive correlation between nuclear parameters and age in our study suggests that nuclear abnormalities increase with age, reflecting disease progression. Ramraj et al.[21] reported a similar trend, showing that nuclear irregularities become more pronounced with advancing age, indicating cumulative exposure to risk factors. Mushi et al.[22] also observed that older women exhibit more significant nuclear atypia, highlighting the importance of regular cervical screening in older populations to detect and manage precancerous changes early.

Our study showed that regular nuclear shape and smooth borders were more common in LSIL, while irregular shapes and borders were predominant in SCC. This progression of nuclear irregularity from LSIL to SCC is consistent with the findings of Ali and Rizvi [23], who noted that nuclear pleomorphism and irregularity are characteristic of malignant transformation. Houston et al.[24] further emphasized that irregular nuclear borders are a hallmark of cancerous cells, supporting our observation that these features are more frequent in SCC.

**Limitations of the Study**

However, there isn't a reference database of morphometric parameters available. Further extensive study is required to determine a range of morphometric parameters for cervical lesions, allowing morphometry to be utilised to improve cervical smear screening accuracy.

**Conclusion**

The present study confirms the significant differences in nuclear morphometric parameters among LSIL, HSIL, and SCC, with SCC showing the most pronounced nuclear abnormalities. These findings align with previous research, highlighting the importance of nuclear morphometry in the screening

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and diagnosis of cervical lesions. Regular screening and early detection, particularly in older women, are crucial for reducing the incidence and mortality of cervical cancer.

**Acknowledgement**

I am immensely grateful to Dr.(Additional Professor.) Ruchi Sinha, Department of Pathology, AIIMS, Patna, Bihar, India, for their support and valuable suggestions.

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