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# EXPLORING CYTONUCLEOMORPHOMETRIC PARAMETERS IN THE DIAGNOSIS OF SQUAMOUS CELL ABNORMALITIES ON LIQUID-BASED CYTOLOGY CERVICOVAGINAL PAP SMEARS: A COMPREHENSIVE EVALUATION

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**ABSTRACT:**

Cervical cancer is one of the leading causes of cancer-related mortality among women globally, particularly in developing countries. This study evaluates the utility of cellular and nuclear morphometry in differentiating squamous cell abnormalities from benign conditions and categorizing these abnormalities on liquid-based cytology cervicovaginal pap smears. The study included 30 cases with confirmed histopathological diagnoses categorized into LSIL, HSIL, and SCC. Significant differences in nuclear area, perimeter, and diameter were found among the groups, enhancing the diagnostic accuracy of cervical cancer screening.

## INTRODUCTION:

Cervical cancer ranks as the second most prevalent cancer among women worldwide and is the most common gynecological cancer in developing countries like India. (1) According to the Global Cancer Observatory, cervical cancer accounts for a significant proportion of cancer-related deaths among women, particularly in regions with limited access to health care and regular screening programs. Public health initiatives have emphasized the importance of early detection and treatment to reduce the incidence and mortality associated with cervical cancer.

Pap smear screening remains the primary method for detecting both precancerous lesions and cervical carcinoma. (2) The introduction of liquid-based cytology has improved the quality of specimens and enhanced the detection rates of abnormal cells. However, the accuracy of cytological diagnosis from pap smears largely relies on identifying characteristic morphological features indicative of dysplastic or malignant cells. Cytological changes resulting from infections, medications, or hormonal fluctuations can closely mimic pre-malignant or malignant alterations in cell morphology, leading to diagnostic challenges and potential false-negative results. (3)

To enhance diagnostic accuracy, nuclear morphometric analysis has been explored as a supplementary tool. Nuclear morphometry involves the quantitative measurement of nuclear features such as area, perimeter, diameter, and compactness. These parameters can provide objective data to distinguish between benign and malignant cells, potentially improving the reliability of cytological assessments. (4)

The significance of accurate cervical cancer screening cannot be overstated, as early detection and treatment are crucial for reducing mortality rates and improving patient outcomes. Understanding the broader context of cervical cancer screening is essential. For instance, integrating universal health coverage (UHC) within the global health security architecture has been identified as a crucial step in pandemic preparedness and response, emphasizing the importance of accessible and equitable health care services. (5) Furthermore, addressing multidrug resistance from a One Health perspective, as highlighted in recent systematic reviews, underscores the interconnectedness of health issues and the need for comprehensive strategies to manage public health threats. (6)

This study aims to assess the utility of cellular and nuclear morphometry in differentiating squamous cell abnormalities from benign conditions and categorizing these abnormalities on liquid-based cytology cervicovaginal pap smears. By integrating morphometric analysis with traditional cytological examination, we hope to enhance the diagnostic accuracy and contribute to better health care outcomes.

**Objectives:**

- To assess the utility of cellular and nuclear morphometry in differentiating squamous cell abnormality from benign conditions.
- To differentiate the categories of squamous cell abnormalities.

## METHODS:

**Study Design and Population:**

This retrospective observational study was conducted in the Department of Pathology at [Institution Name]. The study included cervical pap smears received between August 2023 and December 2023. The inclusion criteria were cases with confirmed histopathological diagnoses categorized as LSIL, HSIL, or SCC based on the Bethesda system. Cases with insufficient clinical data, inadequate smears, or concurrent infections were excluded from the study. A total of 30 cases, with 10 cases each of LSIL, HSIL, and SCC, were included.

**Sample Collection and Preparation:**

Cervical samples were collected using liquid-based cytology (LBC) methods. The cervicovaginal samples were collected using a cytobrush and placed in a vial containing preservative solution. The samples were processed using an automated processor, which ensured even distribution of cells and minimized artifacts. The processed samples were then stained using the Papanicolaou (Pap) staining technique, which highlights cellular and nuclear features essential for cytological evaluation.

**Image Acquisition:**

High-quality images of the stained smears were acquired using a microscope equipped with a 2.5x ocular and a 40x objective. A digital camera attached to the microscope captured the images, which were then transferred to a computer using a frame grabber card. Each case had approximately 25 nuclei analyzed to ensure statistical reliability.

**Nuclear Morphometric Analysis:**

The captured images were analyzed using Image J 1.44C morphometric software, a widely used tool for quantitative image analysis. The nuclear parameters measured included:

- **Nuclear Area**: The total area within the nuclear boundary.
- **Perimeter**: The length of the nuclear boundary.
- **Diameter**: The longest straight line passing through the center of the nucleus.
- **Radius**: The distance from the center to the boundary of the nucleus.
- **Compactness**: A measure of the roundness of the nucleus, calculated as Perimeter2/Area\text{Perimeter}^2 / \text{Area}Perimeter2/Area.

Each parameter was measured in a standardized manner to ensure consistency across cases. The measurements were performed by two independent observers to minimize inter-observer variability.

**Statistical Analysis:**

Data were entered into SPSS software version 25 for statistical analysis. Descriptive statistics, including means and standard deviations, were calculated for each group. ANOVA was used to compare the nuclear parameters

among the three groups (LSIL, HSIL, SCC). The Bonferroni Multiple Comparisons Test was applied post-hoc to determine specific group differences. A p-value < 0.05 was considered statistically significant.

Correlation analyses were conducted to evaluate the relationships between different nuclear parameters. Pearson correlation coefficients were calculated, and scatter plots were generated to visually inspect the linearity and strength of these relationships.

## RESULTS:

The study comprised 30 cases, with 10 cases each of HSIL (Figure 1), LSIL (Figure 1) and SCC . The age distribution of the study population ranged from 30 to 65 years, with a mean age of 45 years. Detailed demographic data are presented in Table 1.

**Table 1: Demographic Data of Study Population**

| Age Group (Years) | LSIL (n=10) | HSIL (n=10) | SCC (n=10) |
|---|---|---|---|
| 30-40 | 4 | 3 | 2 |
| 41-50 | 3 | 4 | 3 |
| 51-60 | 2 | 2 | 4 |
| 61-65 | 1 | 1 | 1 |

**Figure 1:Microscopic image of HSIL**

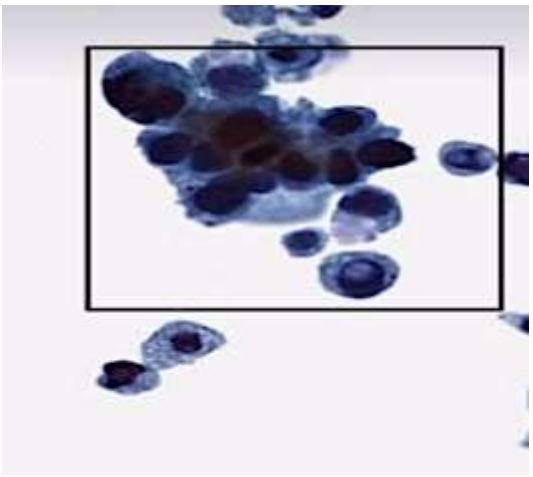
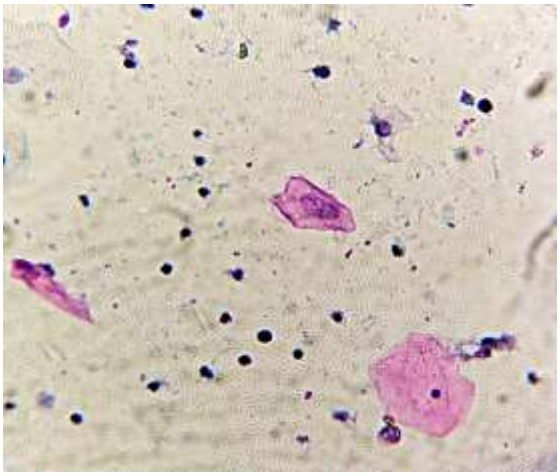


**Figure 2:Microscopic image of HSIL**



### Nuclear Morphometric Analysis

Significant differences were observed in nuclear area, perimeter, and radius between Groups I and II, as well as between Groups I and III. Additionally, there was a significant difference in diameter among all three groups. However, no significant differences were found in nuclear area, perimeter, or radius between Group II and Group III. Furthermore, there were no significant differences in compactness among all three groups.

**Table 2: Nuclear Morphometry Analysis Representation**

| Nuclear Features | LSIL (I) (n=10) Mean +/-SD (range) | HSIL(II) (n=10) Mean +/-SD (range) | SCC(III) (n=10) Mean +/-SD (range) | Anova P value |
|---|---|---|---|---|
| Nuclear Area | 109.54 +/- 11.13 (98.6-120) | 132.7 +/- 17.31 (117-150) | 142.27 +/- 26.62 (116-170) | <0.0001 |
| Perimeter | 24.69 +/-1.143 (23.6 – 25.9) | 27.12 +/- 1.49 (25.4 - 29) | 28.18 +/- 2.72 (25-30.9) | <0.0001 |
| Diameter | 7.72 +/- 0.45 (7.27-8.18) | 8.48 +/- 0.56 (7.72 – 9.09) | 9.06 +/- 0.86 (8.18 - 9.9) | <0.0001 |
| Radius | 3.96 +/- 0.18 (3.7-4.1) | 4.31 +/- 0.29 (4.0-4.65) | 4.53 +/- 0.43 (4.0-4.8) | <0.0001 |
| Compactness | 5.54 +/- 0.045 (5.45-5.63) | 5.56 +/- 0.068 (5.49-5.64) | 5.59 +/- 0.10 (5.59-5.63) | >0.05 |

**Table 3: P Values Between Groups**

| Nuclear Features | Anova | Group I - II | Group I - III | Group II - III |
|---|---|---|---|---|
| Nuclear Area | 0.000005 | 0.00001 | 0.00001 | 0.186 |
| Perimeter | 0.000001 | 0.000001 | 0.0000001 | 0.135 |
| Diameter | 0.0000001 | 0.0000003 | 0.00003 | 0.016 |
| Radius | 0.000002 | 0.00005 | 0.000003 | 0.067 |
| Compactness | 0.123 | 0.280 | 0.056 | 0.287 |

**Correlation Analysis**

Pearson correlation coefficients were calculated to evaluate the relationships between different nuclear parameters. The results are summarized in Table 4.

**Table 4: Pearson Correlation Coefficients Between Nuclear Parameters**

| Parameter 1 | Parameter 2 | Correlation Coefficient | P value |
|---|---|---|---|
| Nuclear Area | Perimeter | 0.85 | <0.0001 |
| Nuclear Area | Diameter | 0.78 | <0.0001 |
| Nuclear Area | Radius | 0.75 | <0.0001 |
| Perimeter | Diameter | 0.82 | <0.0001 |
| Perimeter | Radius | 0.80 | <0.0001 |
| Diameter | Radius | 0.88 | <0.0001 |
| Compactness | Nuclear Area | 0.12 | >0.05 |

## DISCUSSION:

This study provides a detailed evaluation of nuclear morphometric parameters in differentiating squamous cell abnormalities on cervicovaginal Pap smears. Our findings indicate that nuclear area, perimeter, diameter, and radius exhibit significant differences between LSIL, HSIL, and SCC, suggesting that these parameters can serve as reliable markers for identifying and classifying cervical lesions. Specifically, we found that nuclear area and perimeter were substantially larger in HSIL and SCC compared to LSIL, which aligns with the progressive nuclear atypia seen in higher-grade lesions.

The increased nuclear area and perimeter observed in HSIL and SCC can be attributed to the higher mitotic activity and chromatin irregularities characteristic of these lesions. As squamous cells undergo dysplasia and malignant transformation, there is a notable increase in nuclear size and irregularity, reflecting genomic instability and aberrant cell cycle regulation. The significant differences in diameter and radius further underscore the diagnostic value of these parameters, as they reflect changes in nuclear contour and size that are critical for distinguishing between different grades of squamous cell abnormalities.

Interestingly, compactness did not show significant differences among LSIL, HSIL, and SCC. This parameter, which measures the roundness of the nucleus, may not capture the specific morphological changes that occur during the progression from low-grade to high-grade lesions and invasive carcinoma. The lack of significant differences in compactness suggests that while nuclear shape may change, it does not do so in a way that is distinctively measurable by this parameter across the different lesion categories. This finding highlights the need for a multifaceted approach to cytological evaluation, where multiple nuclear parameters are considered together to provide a comprehensive assessment.

Our findings corroborate previous research that has highlighted the importance of nuclear morphometry in cancer diagnostics. For example, studies on breast cancer cytology have shown that increased nuclear area and perimeter are significant predictors of malignancy. (7) Similarly, research on thyroid nodules has demonstrated the utility of nuclear diameter and radius in distinguishing benign from malignant lesions. (8) The consistency of our results with these studies underscores the broad applicability of nuclear morphometric analysis across different types of epithelial cancers.

In the context of cervical cytology, previous studies have reported that nuclear area and perimeter are critical for identifying high-grade lesions. (9) Our study extends these findings by demonstrating that nuclear diameter and radius are also valuable parameters, thereby providing a more detailed morphometric profile of cervical lesions. These results suggest that a combination of nuclear parameters can enhance the diagnostic accuracy of Pap smears, which is particularly important for early detection and treatment of cervical cancer.

The integration of nuclear morphometric analysis into routine cytological examination offers several clinical benefits. Firstly, it provides objective and quantifiable data that can complement traditional cytological assessments, reducing the subjectivity and inter-observer variability that often affect cytological diagnoses. By incorporating these measurements, pathologists can achieve more consistent and accurate diagnoses, which is critical for guiding clinical management and treatment decisions.

Secondly, the use of nuclear morphometry can enhance the sensitivity and specificity of cervical cancer screening programs. Early detection of high-grade lesions and invasive carcinoma is essential for timely intervention, which can significantly reduce cervical cancer morbidity and mortality. By identifying subtle morphological changes that may not be readily apparent through visual examination alone, nuclear morphometry can improve the early detection rates of cervical dysplasia and carcinoma.

Furthermore, the adoption of nuclear morphometric analysis can support public health initiatives aimed at reducing the global burden of cervical cancer. (10) In resource-limited settings where access to advanced diagnostic tools and specialist pathology services is limited, nuclear morphometry can provide a cost-effective and accessible method for improving cervical cancer screening. This aligns with global health goals of enhancing health equity and ensuring universal access to quality healthcare services.

Despite its strengths, this study has several limitations. The sample size was relatively small, comprising only 30 cases, which may limit the generalizability of the findings. Larger, multi-center studies are needed to validate these results and establish robust diagnostic criteria based on nuclear morphometry. Additionally, our study focused exclusively on liquid-based cytology samples. Future research should explore the applicability of nuclear morphometric analysis to conventional Pap smears and other cytological preparations to determine the consistency of these findings across different sample types. Another limitation is the manual nature of the image analysis process, which can be time-consuming and subject to observer bias. The development and validation of automated image analysis systems, incorporating machine learning algorithms, could enhance the accuracy and efficiency of nuclear morphometric analysis. Such systems could rapidly and accurately measure nuclear parameters, reducing the workload for pathologists and minimizing human error.

The broader implications of our findings extend to the field of public health, particularly in the context of cervical cancer prevention and control. Cervical cancer remains a significant health issue globally, with marked disparities in incidence and mortality rates between high-income and low-income countries. Effective screening programs are essential for reducing these disparities and improving health outcomes for women worldwide.

By enhancing the diagnostic accuracy of Pap smears through nuclear morphometric analysis, this study contributes to more effective cervical cancer screening programs. Improved screening can lead to earlier detection of cervical lesions, timely intervention, and ultimately, reduced cervical cancer mortality rates. This aligns with public health initiatives aimed at improving maternal health, reducing mortality rates, and enhancing overall well-being and health outcomes for women globally.

## CONCLUSION:

The nuclear morphometric parameters that significantly differentiated between LSIL and HSIL were nuclear area, perimeter, diameter, and radius. These parameters were also effective in distinguishing between LSIL and SCC, with statistical significance. Specifically, nuclear area, perimeter, and diameter were highly significant in distinguishing between premalignant and malignant cervical smears. However, compactness did not show statistical significance in differentiating between all three groups. Integrating these findings with clinical examination and cytomorphological assessment can enhance the diagnostic accuracy of cervical cancer screening, aiding in the implementation of appropriate treatment strategies. Additionally, methodological approaches in systematic reviews could be employed to further validate and refine nuclear morphometric techniques in the context of cervical cancer screening. (11)

By improving diagnostic accuracy, these methods can contribute to better health care outcomes, reflecting a critical aspect of public health and healthcare. Enhancing early detection and treatment of cervical cancer can lead to improved maternal health, reduced mortality rates, and overall better well-being for women globally.

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