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Role of Morphometry as Diagnostic Adjunct in Evaluating Premalignant and Malignant Cervical Cytology

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Abstract

Background: Malignant lesions of the cervix are the most frequent cause of mortality and morbidity and the third most common cause of cancer deaths in women worldwide. The incidence of cervical cancer is progressively reducing due to the routine use of Papanicolaou (Pap) smears to detect precancerous and early malignant lesions. Moreover, since it is based on subjective morphological assessment, false positive or negative reports are likely to be there. Using morphometric techniques, there have been attempts to use objective parameters to improve the accuracy of reports. In the present study, we used Image Morphometric Software and some of its plugins in order to create macro-images to analyze a large number of cells at a given time and study various nuclear parameters, useful in evaluating pre-malignant and malignant cervical Pap smears.

Method: A retrospective study was done on abnormal Pap smears. Bethesda System was used for the categorization of cervical Pap smears into premalignant and malignant lesions. Nuclear parameters were calculated employing Image-Pro 2.0 Morphometric Software. The analyzed parameters included nuclear area, perimeter, radius, and compactness. The obtained results were statistically analyzed using SPSS software version 19.0.

Results: Nuclear area, perimeter, radius, and compactness were found to be statistically significant parameters in differentiating premalignant from malignant cervical smears ($P < 0.05$).

Conclusion: Nuclear morphometry was found to be a useful objective way and an adjunct to conventional microscopy in differentiating premalignant from malignant cervical lesions.

Keywords: Morphometric analysis, Cervical Pap smears, Nuclear parameters

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Introduction

Cervical cancer is the second most prevalent malignancy occurring among women worldwide. A staggering almost half a million new cases are diagnosed annually.[1] The initial diagnostic technique used in screening for premalignant lesions

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and carcinoma of the cervix is Papanicolaou (Pap) smear examination. It has resulted in a significant drop in the incidence and mortality of cervical cancer by more than 70%.[2] The assessment of the size and diameter of the epithelial nuclei can be done by morphometry of cells found in normal and abnormal smears taken from the uterine cervix. The degree of possible anomalies in these cells can be assessed by analyzing the values of these objective parameters.[3] Cervical cancer begins with a precancerous stage, known as dysplasia, which originates from a normal cell and develops over many years. Cytological evaluation of Pap smear plays a crucial role in the early detection of dysplasia and preinvasive cervical carcinomas.[4]

This study aimed to evaluate the utility of nuclear morphometry in cervical Pap smears to categorize them into premalignant and malignant categories.

Materials and Methods

This study was a retrospective analysis of 100 cervical Pap smears. Ethical clearance was obtained by the Institutional Ethics Committee SHKM GMC, Nalhar Nuh, Haryana, India (EC/0A-19/2017) before commencing the study. Only cases with confirmed histopathology were included. Bethesda system was used to categorize cervical smears into four groups:

Group I –ASCUS (Atypical squamous cell of undetermined significance, n = 26 cases)

Group II –LSIL (Low grade squamous intraepithelial lesion, n = 40 cases)

Group III- HSIL (High grade squamous intraepithelial lesion, n = 26 cases)

Group IV –SCC (Squamous cell carcinoma, n = 8 cases)

Pap smears that did not confirm histopathological diagnosis were excluded from the study. A microscope with a 2.5× ocular and a 40× objective was used to identify a suitable field for analysis with non-overlapping cells. A 640 × 400-pixel digital image of the field was produced by a camera mounted on the microscope and a frame grabber card on the computer attached. The images were stored in the computer memory. A minimum of 25 nuclei per case was analyzed. The measurements were assessed using Image-Pro 2.0 Motic Morphometric Software and subjected to analysis subsequently.

Various nuclear parameters were analyzed:

a) Radius was computed by averaging the length of radial line segments from the center of the nuclear mass to each of the points of the nuclear border.

b) The nuclear area was the area within the outlined nuclear perimeter.

c) The perimeter was measured as the distance around the nuclear border.

d) The compactness of the cell nuclei was calculated using the formula: perimeter^2/ area.[5]

We calculated the mean and standard deviation for all the nuclear features using Microsoft Excel

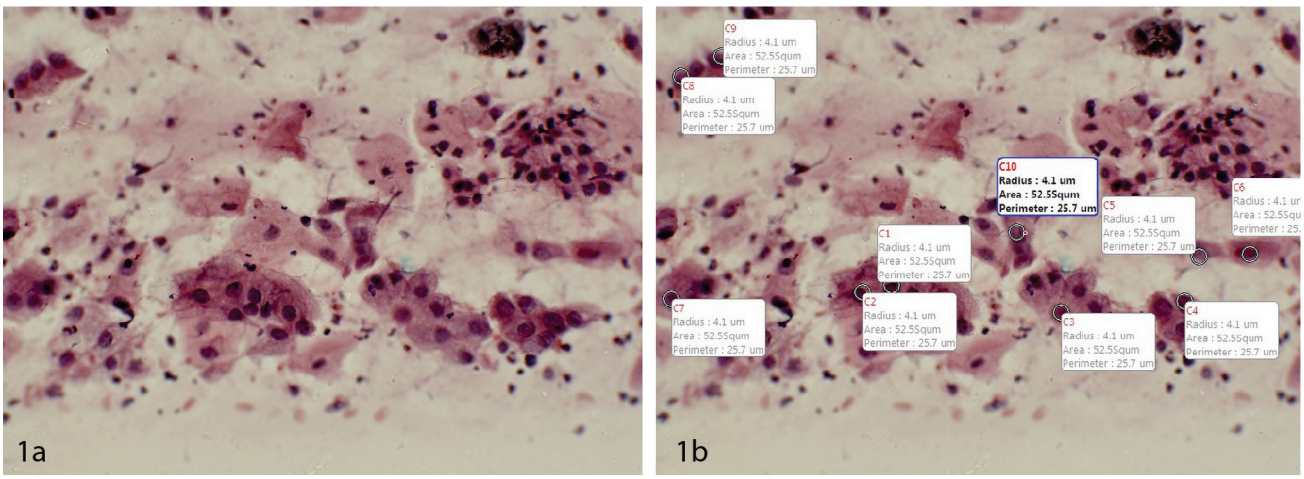

Figure 1. (a, b). Photomicrograph showing atypical squamous cells of undetermined significance (ASCUS) cervix (PAP, 400×) and morphometric parameters (PAP, 400×).

Table 1. The age distribution of different histopathological categories of cervical smears

Age distribution	Group I = ASCUS (n=26)	Group II = LSIL (n=40)	Group III = HSIL (n=26)	Group IV = SCC (n=08)
25- 34 (n=8)	2	2	4	-
35-44 (n=9)	2	2	5	-
45-54 (n=26)	8	18	-	-
55-64 (n=38)	12	12	10	4
65-75 (n=19)	2	6	07	4
Total	26	40	26	08

ASCUS: Atypical squamous cells of undetermined significance; LSIL: Low-grade intraepithelial lesion; HSIL: High-grade intraepithelial lesion; SCC: Squamous cell carcinoma

2016. One -way ANOVA test was applied to determine statistical significance between the groups using SPSS version 19.0.

Results

This study included 100 cases with ages ranging from 24 to 75 years. The peak incidence of neoplastic gynecological lesions (SCC) (8/100; 8.0%) was found among the patients aged 55 to 75 years (Table 1). The presenting complaints included routine cervical cancer screening (60%), abnormal vaginal bleeding (12%), vaginal discharge with strong odor (18%), pelvic pain (12%), and bleeding after sexual intercourse (8%).

Nuclear morphometric analysis was done using Image Pro 2.0 Motic Morphometric Software for image processing and analysis. Table 2 represents the basic results of the study. Group I to group IV increase in the nuclear area, perimeter, radius, and compactness were found between ASCUS and LSIL, LSIL and HSIL, HSIL and SCC, and the difference among the means of each nuclear parameter of these groups was discovered to be statistically significant ($P < 0.05$).

Discussion

Premalignant and malignant lesions of cervix can be treated successfully if diagnosed early. Early diagnosis can be done by Pap Smear examination. It is useful in picking up abnormal cells from precancerous lesions as well as malignant ones. In the present study, all the four nuclear morphometric parameters studied (nuclear area, radius, perimeter, and compactness) could be used to differentiate between premalignant and malignant cervical smears. These parameters showed significant differences among ASCUS, LSIL, HSIL, and SCC with P-value < 0.0001 (Figures 1-4). The cells belonging to each diagnostic category could be identified on the basis of the morphometric characteristics measured.

Routine cervical cancer screening has remarkably decreased the morbidity and mortality from newly-diagnosed cervical cancer cases each year. The Pap test is a simple, quick, and painless screening test on cells scraped from the uterine cervix. George Papanicolaou introduced it as a screening technique for cervical cancer in 1943

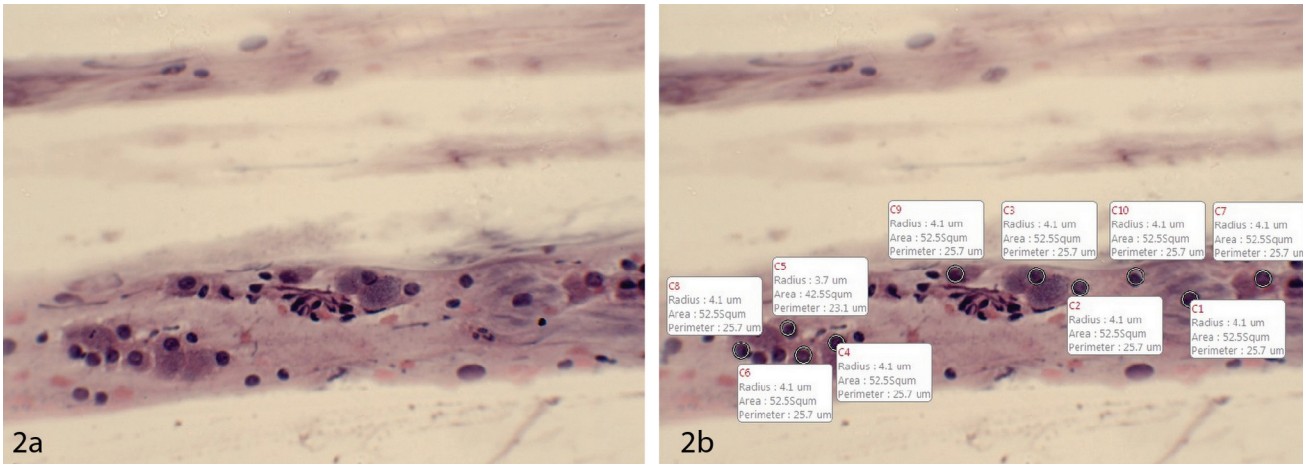

Figure 2. (a, b). Photomicrograph showing low-grade squamous intraepithelial lesion (LSIL) cervix LSIL (PAP, 400×) and morphometric parameters (PAP, 400×).

and it is named after him.[6]

To standardize cervical cytology reporting, the Bethesda System (TBS) formulated a uniform reporting format in 1988 and the concept of ASCUS was introduced. However, it proved to be controversial for clinicians and pathologists, highlighting the ingrained drawbacks of subjective morphologic interpretation. With TBS terminology becoming popular in the early 1990s, it was found that most of the abnormal cervical cytology results were categorized into ASCUS or LSIL.[7]

An objective analysis of cervical cytology by automating the microscopic analysis of cervical cells was explored using computer vision techniques. The classification of abnormal cells was done by the cell segmentation technique. However, due to cell overlapping and variability of color and intensity, accurate segmentation of cytoplasm is difficult.[8] This has led to the exploration of nuclear morphometry as a diagnostic option.

Rani et al. found a gradual increase in the nuclear area and perimeters in carcinoma when contrasted with premalignant lesions. Nuclear area, perimeter, diameter, and radius were the morphometric parameters that could significantly differentiate between LSIL and HSIL and also LSIL and SCC.[9] These parameters were highly notable in differentiating premalignant from malignant cervical smears ($P < 0.0001$). Compactness was not found statistically significant in differentiating between these groups ($P > 0.05$). This is in partial concordance with our study, where we found that the nuclear area, radius, perimeter, and compactness were significant in differentiating between premalignant and malignant cervical smears.

They also found nuclear morphometry to be a useful objective technique for differentiating between premalignant and malignant cervical cytology. It was found to be useful in navigating diagnostic gray zones on cervical smears, particularly pertaining to ASCUS or AGUS. They opined that by combining the findings of clinical examination, cytomorphological, and nuclear morphometric parameters, the diagnostic accuracy of cervical cancer screening can be greatly enhanced.[9]

Normal cells are differentiated from abnormal ones based on the change in nuclear size, irregularity of nuclear shape, and granularity of nuclear chromatin. These criteria are evaluated subjectively by cytopathologists. In a PC-based study on Cytopathologic Image Analysis System and Support Vector Machine (SVM), it was demonstrated that perimeter, nuclear area, maximum length, maximum width, and nuclear-cytoplasmic (N/C) ratio in dysplastic cells were significantly higher vis-a-vis benign cells on statistical examination (P value 0.001).[4]

The software used by Sanyal et al. in a pilot study of an open-source image analysis software for automated screening of conventional cervical smears was able to distinguish between benign

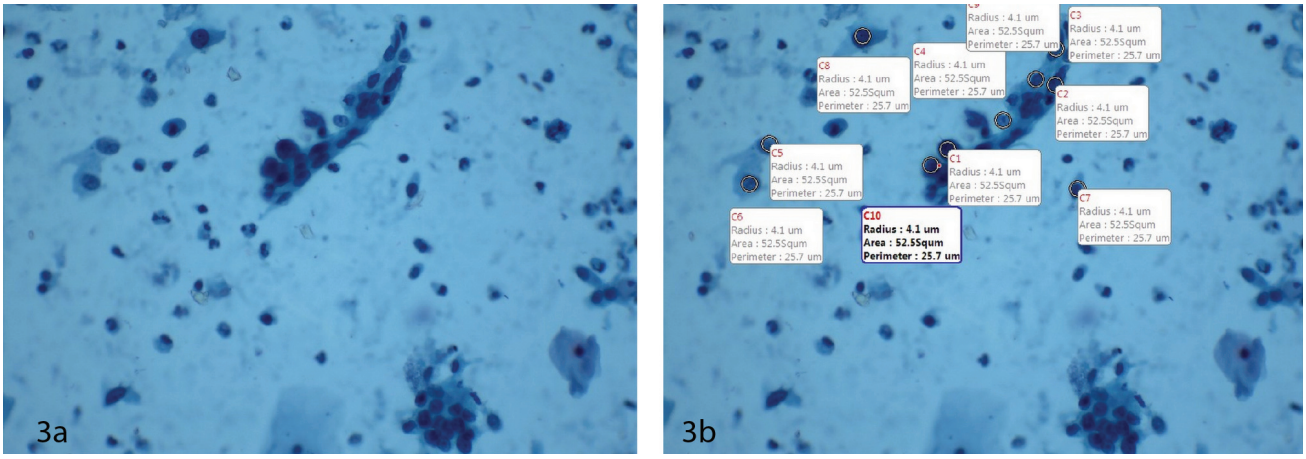

Figure 3. (a, b). Photomicrograph showing high grade squamous intraepithelial lesion (HSIL) cervix (PAP, 400×) and morphometric parameters(PAP, 400×).

Table 2. Average nuclear parameters values among various histopathological groups of cervical smears

Nuclear features	ASCUS±SD (n=26)	LSIL±SD (n=40)	HSIL±SD (n=26)	SCC±SD (n=8)	*P* value
Nuclear area	98.25 ± 0.80	110.2 ± 2.39	137.4 ± 2.5	150.35 ± 4.30	0.000*
Perimeter	22.36 ± 0.64	25.7 ± 1.22	27.9 ± 0.86	29.02 ± 0.94	0.000*
Radius	03.26 ± 0.14	03.9 ± 0.33	04.3 ± 0.21	04.4 ± 0.22	0.000*
Compactness	5.09 ± 0.27	5.99 ± 0.45	5.67 ± 0.32	5.60 ± 0.32	0.000*

*Statistically significant; ASCUS: Atypical squamous cells of undetermined significance; LSIL: Low-grade intraepithelial lesion; HSIL: High-grade intraepithelial lesion; SCC: Squamous cell carcinoma

and malignant foci with approximately 68% sensitivity with a combination of parameters. They found the specificity to be 80% with a significant number of false positives.[10]

Sindhu et al. studied nuclear morphometry of abnormal Pap smears. They found parameters like area, perimeter, and maximum diameter to be significantly different between LSIL, HSIL, and SCC. This was in concordance with three of the four parameters studied by us. They did not study compactness and hence, it cannot be compared to the present study. Additionally, the mean values for the nuclear area, perimeter, and the maximum diameter of SCC smears were found to be lower than those in the HSIL group. They also opined that the higher standard deviation in the SCC smear group indicated greater anisonucleosis and concluded that anisonucleosis detected by nuclear morphometry is a better marker of neoplasia than the changes in the dimensions of nuclei.[11]

Tiwari et al. evaluated the role of nuclear morphometry in the screening of cervical Pap smears. They found a gradual increase in the nuclear area, diameter, and N:C ratio comparing normal cells to dysplastic ones and SCC. On the other hand, the mean cell area and the mean cell perimeter were found to decrease gradually for all the lesions except for ASC-H compared with LSIL and HSIL. All the five parameters studied by them were found to be statistically significant ($P < 0.001$) in differentiating among the groups studied.[12] This is up to an extent different from our findings, where we found a gradual increase among all the parameters studied across the groups with an added parameter of compactness giving significant results when compared among the groups studied.

Morphometry has been explored as an adjunctive diagnostic tool in other areas of diagnostic pathology, including thyroid and breast. Nuclear morphometry, including texture analysis, has been found to be of utility in assisting in the cytologic diagnosis of thyroid lesions.[13] Furthermore, in breast cytodiagnosis, morphometry has been used to successfully differentiate between benign and malignant aspirates, including navigating gray areas viz

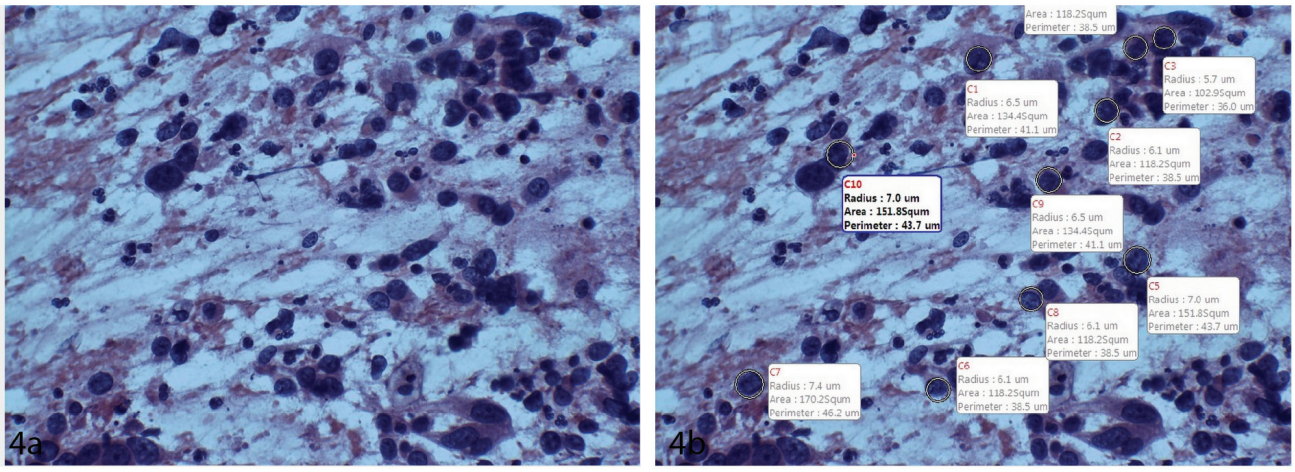

Figure 4. (a, b). Photomicrograph showing squamous cell carcinoma (SCC) cervix (PAP, 400×) and morphometric parameters (PAP, 400×).

atypical ductal hyperplasia and ductal carcinoma in situ. It has also been found to correlate significantly with cytologic grades.[14]

To summarize the key results of the present study vis-a-vis the study objectives, statistically significant differences were discovered in nuclear morphometric parameters studied viz the nuclear area, radius, perimeter, and compactness in cervical carcinoma as compared with premalignant lesions on Pap smear examination. All these four parameters were useful to differentiate between ASCUS, LSIL, HSIL, and SCC and the difference among these parameters was statistically significant.

A significant limitation of the present study is the manual marking of the nucleus for morphometry, which is fraught with the risk of human error. Additionally, further studies with a bigger sample size will give more clarity on the results obtained in this study.

A possible source of potential bias could be that these measurements have been assessed as a diagnostic adjunct to subjective cytology in already diagnosed and proven cases.

Conclusion

Cervical screening using Pap smear examination is widely recognized and accepted in differentiating abnormal cells from normal ones. However, false-negative results due to subjective interpretation may unnecessarily postpone the required treatment. Likewise, false-positive results can create havoc. The morphometric characteristics of cervical cells will prove to be a useful adjunct in reaching an accurate diagnosis of preneoplastic and neoplastic lesions and in differentiating among them objectively.

Conflict of Interest

None declared.

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