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Original Research Article

Nuclear Morphometric Analysis of Exfoliated Cells and its Utility in Diagnosing Cervical Lesions – Our Experience in a Tertiary Care Center

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Abstract:

Nuclear morphometric analysis of exfoliated cells and its utility in diagnosing cervical lesions – our experience in a tertiary care center.

Background: Cervical cancer is the fourth most common cancer affecting women worldwide . A pap test is an important screening procedure for cervical cancer and accuracy depends on morphological features of dysplastic or malignant cells. Morphometric analysis of nuclear parameters like area, perimeter, maximum diameter and nuclear compactness are important to determine dysplastic cells which increase the sensitivity of pap smear results. Our present study aim to evaluate the nuclear morphometry in LSIL, HSIL and squamous cell carcinoma in pap smear.

Materials and Methods: 80 cases were selected which were diagnosed as LSIL, HSIL, squamous cell carcinoma and normal pap smears as control. Representative areas of the smear were selected and digital images were produced by camera on the microscope using 40X objective and were analyzed through software ProgRes Capture Pro2.8.8. Measurements of nuclear parameters like nuclear area, perimeter, maximum diameter and compactness (perimeter^2/area) were made on the cell images.

Results: The nuclear parameters of neoplastic cells were more than those of normal cells. Nuclear area, perimeter, diameter were found to be statistically significant ($p<0.05$) parameters in differentiating premalignant from malignant cervical smears.

Conclusion: Nuclear morphometry can be used as a diagnostic tool and will increase sensitivity in differentiating between pre-malignant and malignant lesions of cervix.

Keywords: Nuclear perimeter, LSIL, HSIL, pap smear, ProgRes software.

Introduction

Cervical cancer is the fourth most common cancer affecting women worldwide, now the second most common cancer in India[1]. The incidence of cervical cancer has been declining in last three or four decades in most developed countries due to introduction of cervical screening programmes. A pap test is an important screening procedure for cervical cancer. The identification of precancerous lesions by the pap test decreases the occurrence of precancerous lesions and avoids the development of invasive cancer[2].

The background of pap test, developed by George Papanicolaou, father of cytology, has shown that specific cervical cells have morphological characteristics that can be used to diagnose carcinoma. Cells are obtained from the cervix, the lower part of the uterus protruding into the vagina[3]. The most mature squamous cell is considered a superficial cell, typically polygonal (45-50µm in diameter) with a pyknotic nucleus (5-6µm). Intermediate cells are also mature squamous cells, polygonal (20-4-µm) with vesicular nucleus (6-9µm). the immature cells are parabasal and basal

cells, parabasal cells are circular or oval (15-30µm), nucleus is variable in size larger than that of intermediate cell. Parabasal and basal cells are not normally seen in pap smear because it does not scrape off entire thickness of the epithelium[4].

The Bethesda system for reporting the results of cervical cytology was developed as a uniform system of terminology that would provide guidance for clinical management. The 2014 Bethesda system terminology reflects important advances in biological understanding of cervical neoplasia and cervical screening technology[5].

Low grade squamous intra-epithelial neoplasia (LSIL): squamous cells arranged in sheets or singles with nuclear enlargement , hyperchromasia and koilocytic change.

High grade squamous intra-epithelial neoplasia (HSIL): Squamous cells in singles, sheets and syncytial like with hyperchromatic nuclei and high N:C ratio and coarse chromatin.

Squamous cell carcinoma (SCC): Squamous cells show marked variation in size and shape with pleomorphic nuclei, prominent macronucleoli, coarse chromatin and orangeophilic cytoplasm, may be associated with tumor diathesis [6].

Pap smears have 98% specificity and 51% sensitivity of diagnosing the cervical lesions. Inflammatory conditions mimic the features of malignancy [7]. Nuclear morphometric features like nuclear area, perimeter, diameter and compactness may improve the efficiency of the cytology.

Many studies are conducted in analyzing the nuclear features by morphometry in lesions of breast [8], exfoliated cells of buccal mucosa [9], cervix [10,11]. The present study is aimed to evaluate the nuclear morphometric features of cervical pap smears in LSIL, HSIL and SCC and compare with nuclear morphometric features of normal smears there by increasing the sensitivity of pap smears.

Material and Methods

A retrospective study of biopsy proven cervical pap smears reported as LSIL, HSIL and squamous cell carcinoma carried out in the department of Pathology of a tertiary care hospital from December 2022 to October 2023. Sample size was 80cases. 30cases (biopsy proven LSIL, HSIL, SCC cases 10 each) and 50 cases of normal pap smears taken as control for the study.

Inclusion Criteria:

Cases which were screened by routine pap smears and diagnosed as LSIL, HSIL and SCC and confirmed by histopathological diagnosis were chosen for the study.

Exclusion Criteria:

1. Atypical glandular cells of undetermined significance (AGUS), Atypical squamous cells of undetermined significance (ASCUS), Atypical squamous cells –cannot rule out HSIL (ASC-H), inflammatory smears.

2. Unsatisfactory Smears.

Papanicolaou stained cervical smears were screened and representative areas i.e., where the cells are distributed in a monolayer pattern without overlapping of cells, were selected and digital images were taken by ProgRes Camera and nuclear morphometric parameters (nuclear diameter, area, perimeter and compactness: $Perimeter^2/area$) were measured using software ProgRes Capture Pro2.8.8. a) Nuclear diameter (ND): Diameter was the diameter of the circle with the same area as the outlined nucleus. b) Nuclear area (NA): Area within outlined nuclear perimeter (NP) c) NP: Distance around nuclear border d) Nuclear compactness (NC): $Perimeter^2/area$

Ethics

Institutional ethical approval has been taken. Approval No. Faculty/1047/23.

Results:

Data was entered in MS excell and analyzed using SPSS trial version21. Continuous variables were analyzed using ANOVA test and student t test.

Results the sample size included 80 cases with 50 normal pap smears and 30 abnormal smears comprising LSIL, HSIL and SCC 10 cases each

Table 1: The age distribution of the cases

Age in years	Normal pap smears (n=50)	LSIL (n=10)	HSIL (n=10)	SCC (n=10)
20-30	15	1	-	-
31-40	12	4	1	-
41-50	16	2	3	2
51-60	5	2	4	3
61-70	2	1	2	5
	50	10	10	10

The nuclear morphometric analysis of normal pap and abnormal pap smears were done. The results were obtained in µm. The mean values for the individual cases were calculated and documented in master chart. The mean values along with standard deviations obtained for all cases of normal pap smears and abnormal pap smear groups and comparision was done.

50 normal pap smears were taken as control. In each of the smears 50 nuclei were assessed and morphometric analysis of nuclear parameters like area, perimeter, maximum diameter and compactness were taken and mean taken for that smear. Mean with standard deviation for 50 cases was calculated for each nuclear parameter. The same was done for 30 abnormal cases. Table 2.

Table 2: Mean with standard deviation for Normal and abnormal pap smears.

Variables	Normal (Mean±SD)	LSIL (Mean±SD)	HSIL (Mean±SD)	SCC (Mean±SD)
Diameter in microns	6.78±1.17	10.84±0.51	12.81±0.44	15.74±0.39
Perimeter	23.32±4.92	34.7±1.64	48.62±1.2	64.92±2.53
Area	33.14±9.49	81.12±4.44	116.71±6.73	147.02±2.21
Compactness	16.95±5.1	14.89±1.37	20.33±1.63	28.68±1.88

In the present study we compared the nuclear parameters between normal and abnormal pap smears using ANOVA Table 3.

Table 3: Nuclear morphometric analysis between normal and abnormal pap smears

Variables	Normal (Mean±SD)	LSIL (Mean±SD)	HSIL (Mean±SD)	SCC (Mean±SD)	F value	P value
Diameter in microns	6.78±1.17	10.84±0.51	12.81±0.44	15.74±0.39	305.42	<0.001
Perimeter	23.32±4.92	34.7±1.64	48.62±1.2	64.92±2.53	342.75	<0.001
Area	33.14±9.49	81.12±4.44	116.71±6.73	147.02±2.21	738.82	<0.001
Compactness	16.95±5.1	14.89±1.37	20.33±1.63	28.68±1.88	24.83	<0.001

We compared the nuclear parameters between normal and individual abnormal pap smears using student t 'test Table 4.

Table 4: P value between normal and abnormal pap smears

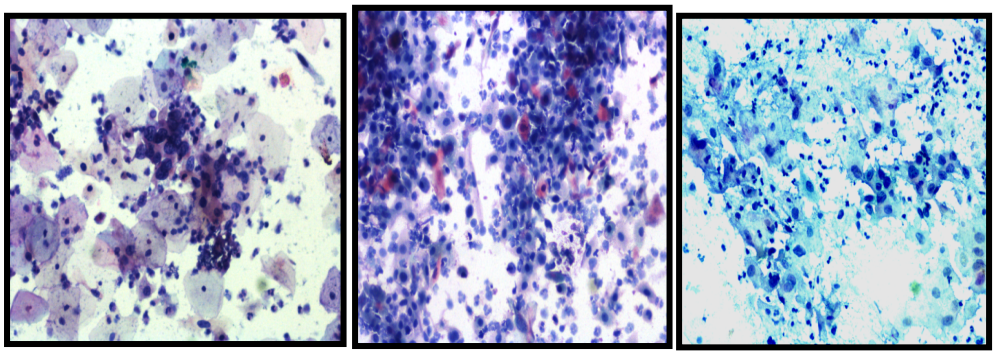
Nuclear parameters	P Value		
	Normal & LSIL	Normal & HSIL	Normal & SCC
Diameter in microns	<0.001	<0.001	<0.001
Perimeter	<0.001	<0.001	<0.001
Area	<0.001	<0.001	<0.001
Compactness	0.02	<0.001	<0.001

We compared the nuclear parameters between abnormal groups using student 't test Table 5.

Table 5: Comparison of p values between the abnormal pap smear groups in present study

Nuclear parameter	P value		
	LSIL - HSIL	LSIL - SCC	HSIL - SCC
Diameter in microns	<0.001	<0.001	<0.001
Perimeter	<0.001	<0.001	<0.001
Area	<0.001	<0.001	<0.001
Compactness	<0.001	<0.001	<0.001

The nuclear parameters were found to be statistically significant (p value<0.001) between normal and abnormal smears and also between abnormal smears.



LSIL Pap stain 40X **HSIL Pap stain40X** **SCC Pap stain 40X**

Discussion

Cervical cancer can have devastating effects with a very high human, social and economic cost, affecting women in their prime. Low-tech and inexpensive screening tools exist and could significantly reduce the burden of cervical cancer

deaths right now in less developed countries[12]. Educational level, awareness about pap smear test, treatability of cervical cancer and preventability of cervical cancer are factors that showed a statistically significant relationship with utilization of pap smear test [13].

Many reactive, infectious and inflammatory conditions give rise to cells which mimic malignancy and lead to false positive results in the patient which have an impact on the management of disease. Cytological criteria for epithelial abnormalities is mainly subjective but in computed morphometry , the nuclear parameters can be easily analyzed thus minimizing the false positive results[11]. The precise categorization into pre-malignant or malignant on microscopic examination is highly subjective. Morphometric analysis of nuclear parameters like area, perimeter, maximum diameter and nuclear compactness are important to determine dysplastic cells which increase the sensitivity of pap smear results.

The present study deals with the morphometric assessment of nuclear parameters like area, perimeter, maximum diameter and compactness using software ProgRes Capture Pro2.8.8. In the present study, we had confirmed histopathological diagnosis for all selected abnormal cervical pap smears. LSIL, HSIL and SCC cases were given cervical intraepithelial neoplasia CINII/III and squamous cell carcinoma on histopathology respectively.

In the present study, nuclear size related parameters (area, perimeter, maximum diameter) and compactness of nucleus were appropriate parameters to differentiate between normal pap from abnormal pap smears. These parameters showed significant differences between normal and abnormal pap smears which was highly significant with p value <0.001. the nuclear dimensions of cells in abnormal pap smears were in general significantly much higher than the corresponding nuclear measurements in normal smears which were concurrent with the studies conducted by Vijayashree R et al and Dr. Sindhu C et al. In study conducted by Vijayashree R et al, they analyzed nuclear morphometry in inflammatory and ASCUS smears. Their study showed anisonucleosis, to be a better indicator of neoplasia[10]. In study conducted by Dr. Sindhu C et al, they analyzed nuclear morphometry in LSIL,HSIL and SCC whose results are in concordance with those of our study.

In the present study, the nuclear parameters showed significant difference between LSIL, HSIL and SCC which was highly significant with p-value <0.001 that showed similar results when compared with the study conducted by Divyarani et al [11]. In a study done by Huang et al., on cervical smears by PC based cytopathologic image analysis system and support vector machine showed that in dysplastic cells, the morphometric parameters like perimeter, nuclear area, maximum length, maximum width, N/C ratio were all found to be statistically significant with p- value of 0.001. our study results in relation to nuclear area, perimeter and maximum diameter are in concordance with Huang et al study[14].

The present study showed there was a gradual increase in nuclear area, perimeter, diameter and compactness in carcinoma when compared to premalignant lesion (LSIL and HSIL).

Laishram S and Shariff S studied the nuclear morphology with regard to nuclear diameter; nuclear area; coefficient of variation of nuclear area; nuclear/cytoplasmic ratio and the ratio of largest to smallest nuclear diameter (L:S ratio) on 60 breast FNAC and found nuclear parameters to be significantly higher in the malignant lesions when compared to benign lesions [15] .

Kashyap et al. studied. Nuclear morphometry on cytology of benign and malignant breast lesions and found that nuclear morphometry could differentiate between benign and malignant aspirates with a gradually increasing nuclear size parameters like nuclear area, equivalent diameter, minimum feret, maximum feret, and perimeter [16]

Prasad H et al., studied morphologic and cytomorphometric analysis of exfoliated buccal mucosal cells in 50 diabetic patients with 5 controls. Smears were stained by Papanicoloau method and using a micrometer mean values of nuclear diameter, cell diameter, cytoplasmic diameter and nucleus: cytoplasmic ratio were obtained and found that diabetes produces definite morphological and morphometric changes in exfoliated buccal cells [9] .

Mudaliar K and Hutchens K studied morphometric image analysis of 60 cases of Irritated Seborrheic Keratoses, Verruca Vulgaris, Hypertrophic Actinic Keratoses and Squamous Cell Carcinoma (15 cases of each) and found statistically significant differences in nuclear size and cellularity between the benign and the pre-malignant and malignant neoplasms [17] .

The results of nuclear morphometry using various parameters like area, perimeter, diameter, radius and compactness can be made even more precised using automated methods for screening of Pap smears with help of smart image recognition software which can automatically select the correct boundary of a nucleus [18] .

Nuclear morphometry can also be utilised as a diagnostic tool especially in gray zones [16] on cervical smears, especially by ASCUS or AGUS which are encountered during reporting of Pap

smears [11] . We found that most widely used parameters in various studies done on morphometry include mean nuclear area, perimeter and diameter. In the present study we found nuclear area, perimeter and diameter to be of more significance in differentiating the premalignant and malignant lesions. The study by Narasimha A et al. [19] also showed compactness to be a significant nuclear parameter between the benign and malignant conditions in their study.

Conclusion

The present study showed that the nuclear morphometric parameters related to nuclear size like area, perimeter and diameter were significantly larger in abnormal pap smears than the normal smears. We can conclude that in pap smears, anisonucleosis detected by nuclear morphometry is a better marker of neoplasia than the changes in the dimensions of the nuclei.

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