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Role of nuclear morphometry in cervicovaginal Papanicolaou smears and its utility in diagnosing cervical lesions

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Abstract
Background: Pap smears are considered as the primary diagnostic modality in detecting the pre-malignant lesions and Carcinoma cervix. Cellular changes due to infections, drugs, hormonal fluctuations mimic pre-malignant conditions which results in false negative results. Hence nuclear features are very important for diagnosing dysplastic cells. The nuclear parameters like Area, Perimeter, Maximum diameter, Radius and Nuclear compactness are important to determine dysplastic cell by morphometric analysis which increase the sensitivity of the results of Pap smears.
Objectives: (1) To study the conventional Pap smears findings of biopsy proven cases of Low grade Squamous Intraepithelial Lesion (LSIL), High grade Squamous Intraepithelial Lesion (HSIL) and Squamous Cell Carcinoma (SCC).
(2) To study the nuclear morphometric parameters using Image J software and correlate the findings of Pap smear cytology with the nuclear morphometric parameters.
Methodology: 125 cases were selected retrospectively which were screened by Routine pap smears and diagnosed as LSIL (25 cases), HSIL (25 cases), Squamous cell carcinoma (25 cases) and Normal Pap smears (50 cases) were included as control. Representative areas of the smear were selected and digital images were produced by a camera on the microscope using 40x objective and the images were analyzed through Image J Software developed by National Institute of Health, USA. Around 50 nuclei/smear were analyzed and measurements of nuclear parameters like Nuclear Area, Perimeter, Maximum diameter, Radius and Compactness (perimeter^2/area) were made on the cell images in a precise manner.
Results: The size of neoplastic nuclei were larger than normal nuclei. More than the enlargement, anisonucleosis was found to be a better indicator of neoplasia.
Conclusion: Nuclear morphometry can be used as a diagnostic tool in differentiating between the premalignant and malignant lesions of cervix.

Keywords: LSIL, HSIL, SCC, Nuclear morphometry, Image J software.

Introduction
Cervical cancer is the fourth most common cancer affecting women worldwide, the most common cancer in women in several less developed countries, and now the second most common cancer in India [1].
Cervical cancer continues to be the most common genital cancer encountered in clinical pattern in India. 5 lakh new cases of cancer cervix are reported annually world over. Cervical cancer accounts for 15% of all cancers in females [2]. In a study conducted by Nair MK *et al*. found cervical cancer to be most common cancer in females in India, 2015 which accounted for 1, 39864(26.1%) incident cases [3].
Cervical cancer is less common in Muslim than in Hindu women. Rural women are at higher risk of developing cervical cancer as compared to their urban counterparts and is the third largest cause of cancer mortality in India accounting for nearly 10% of all cancer related deaths in the country [4]. About 5.0% of women in the general population are estimated to harbour cervical HPV-16/18 infection at a given time, and 83.2% of invasive cervical cancers are attributed to HPVs 16 or 18 [5]. The age-adjusted incidence rate of cervical cancer varies widely among registries; highest is 23.07/100,000 in Mizoram state and the lowest is 4.91/100,000 in Dibrugarh district [6]. The recent NCRP data show that between 2009 and 2011 Aizawl district in the north eastern part of India had the highest levels of cervical

Cancer at an age-adjusted rate of 24.3, followed by Barshi at 19.5 and Bangalore at 18.9 [7, 8].

In India, Carcinoma cervix remains the most common type of cancer in women [9]. The incidence of cervical cancer has been declining in last three or four decades in most developed countries predominantly due to the introduction of cervical screening programmes. No form of cancer documents the remarkable effects of screening, early diagnosis and curative therapy on the mortality rate than does the cancer of cervix [10]. The introduction of cervicovaginal cytology as a means to detect precancerous lesions of the uterine cervix has been milestone in the study of cancer of uterine cervix [11].

The Bethesda System for reporting the results of cervical cytology was developed as a uniform system of terminology that would provide clear guidance for clinical management. The 2014 Bethesda system terminology reflects important advances in biological understanding of cervical neoplasia and cervical screening technology [12].

Pap smears have 98% specificity and 51%sensitivity of diagnosing the cervical lesions. Inflammatory conditions mimic the features of malignancy [13]. It is important to differentiate the nuclear features in inflammatory and malignant conditions, which increases the sensitivity of the Pap smears.

However many studies are conducted in analyzing the nuclear features by morphometry in lesions of breast, [14] exfoliated cells of buccal mucosa [15] cervix [16, 17].

In the present study, nuclear morphometric features of cervical pap smears are analyzed using Image software in pre-malignant and malignant conditions thereby increasing the sensitivity of Pap smears.

Objectives

1. To study the conventional Pap smears findings of biopsy proven cases of Low grade Squamous Intraepithelial Lesion (LSIL), High grade Squamous Intraepithelial Lesion (HSIL) and Squamous Cell Carcinoma (SCC).
2. To study the various nuclear morphometric parameters of the corresponding conventional Pap smears by using Image J Software and correlate the findings of Pap smear cytology with nuclear morphometric parameters of the Pap smears.

Methodology

Source of data

The study was a retrospective study of histopathology findings of cervical biopsies and corresponding cervical Pap smears carried out in the Department of Pathology, MVJ Medical College and Research Hospital, Bangalore for a period of 4 years, from September 2013 to August 2017.

Sample size: 125 cases (LSIL, HSIL and Squamous Cell Carcinoma include biopsy proven cases) and 50 cases of Normal Papanicolaou smears were taken as control for the study.

Inclusion criteria

1. Cases which were screened by Routine pap smears and diagnosed as LSIL, HSIL and Squamous cell carcinoma.
2. LSIL, HSIL and SCC of confirmed histopathological diagnosis are chosen for the study.

Exclusion criteria

1. AGUS, ASCUS, ASC-H, Inflammatory smears
2. Unsatisfactory smears.

Methodology

Cases between September 2013 to August 2017 were considered for the study. The Histopathology findings of cervical biopsies of LSIL, HSIL and SCC were reviewed and the corresponding Pap smears were screened and classified according to the Bethesda System 2014.

The Pap smears and the corresponding resected specimens and cervical biopsies were stained with Papanicolaou stain and Hematoxylin and Eosin stain respectively in all cases. Rapid Pap stain by RAPID-PAPTM [PAPANICOLAOU STAIN KIT] which was commercially available from Biolab Diagnostics Private Limited, Boisar, India was used to stain Pap smears.

The cervical biopsies and hysterectomy specimens for histopathological examination were fixed in 10% formalin. Cervical biopsies were sliced and taken in Toto. Representative bits were taken from hysterectomy specimens. These bits were placed in cassettes and kept in fixative and processed in the automatic tissue processor for 16hrs.Paraffin tissue blocks were prepared and 3-4micron thick sections were cut and stained with routine hematoxylin and eosin stain.

The Pap smears were scanned with scanner and 10x objectives of the microscope. Representative areas of the smear i.e. where the cells are distributed in a monolayer pattern without overlapping of cells were selected and digital images were produced by a camera on the microscope using 40x objective. Nuclear analysis was done in an area equivalent to 4, 75, 0000 (2500x1900) pixels.

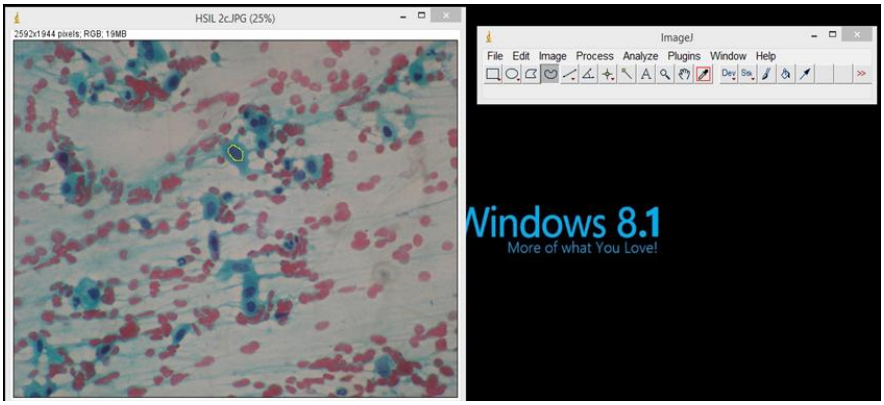

Fig 1: Nuclear morphometry image analysis using Image J software

The morphometric analysis was done using Image J Software developed by National Institute of Health, USA. The Image J software 1.50a / Java 1.8.0_51 (32 bit) was downloaded in the hp laptop. Around 50 nuclei/smear were analyzed and measurements of nuclear parameters that is Nuclear Area, Perimeter, Maximum diameter, Radius and Compactness (perimeter^2/area) were made on the cell images in a precise manner using the software.

1. Nuclear area was the area within the outlined nuclear perimeter
2. Perimeter was measured as the distance around the nuclear border
3. Diameter was the diameter of the circle with the same area as the outlined nucleus. In our study we considered Feret diameter which was the longest distance between two points on the selection boundary.
4. Radius 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
5. Compactness of the cell nuclei calculated using the formula: perimeter^2 / area

Statistical Analysis

The results obtained by computerized Cyto morphometry were compared between the normal Pap smears and abnormal Pap smears groups and also between the abnormal Pap smear groups. The most distinctive morphometric features among the various nuclear morphometric features were analyzed with the data obtained. The nuclear parameters between the normal Pap smears and abnormal Pap smear groups were compared using Student t test, between the abnormal groups using ANOVA and the nuclear parameters between the abnormal Pap smear groups were compared using Student t test. Statistical analysis was performed using Free analysis of Variance statistical software. Comparisons between the individual groups in the abnormal Pap smears were also analyzed using post hoc test i.e. Bonferroni Multiple Comparisons Test.

A p-value < 0.05 was considered as statistically significant.

Results

The sample size included 125cases with 50 normal Papanicolaou smears and 75 abnormal Papanicolaou smears comprising of LSIL, HSIL and SCC 25 cases each. The abnormal Pap smears were again categorized into 3 groups as Group I – LSIL, Group II – HSIL and Group III – SCC. The age distribution of the cases is shown in Table 1. The clinical diagnoses included routine screening, bleeding per vagina, white discharge per vagina and growth in the cervix.

Cytological features are as follows (Fig 2)

LSIL- Squamous cells arranged in sheets or singles with nuclear enlargement, hyperchromasia and koilocytic change

HSIL – Squamous cells in singles, sheets and syncytial like with hyperchromatic nuclei and high N: C ratio and coarse chromatin

SCC – Squamous cells displaying marked variation in size and shape with pleomorphic nuclei, prominent macronucleoli, coarse chromatin and orangeophilic cytoplasm may be associated with tumor diathesis.

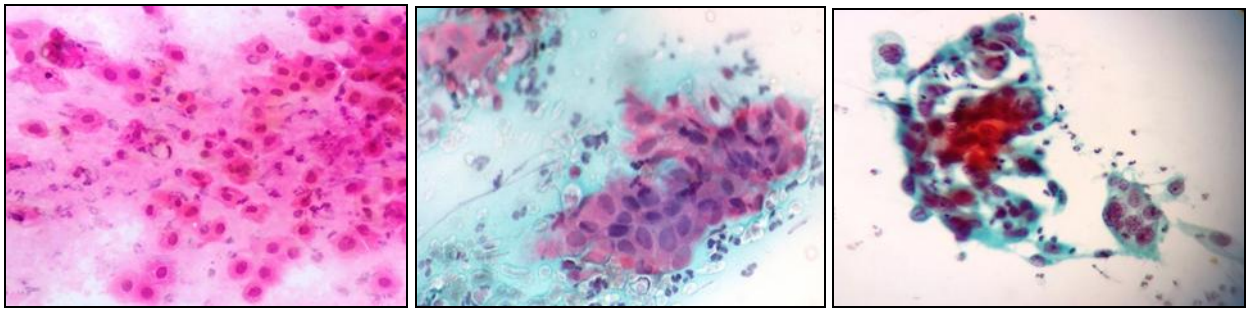

Fig 2(a, b, c): [Pap stain, Conventional Preparation, 400x]:(a) LSIL- cells in sheets with N:C ratio, hyperchromatic nuclei and inconspicuous nucleoli. (b)HSIL-Synctial like aggregates with hyper chromatic nuclei and delicate cytoplasm. (c)SCC-cells in clusters with nuclei displaying coarse granular chromatin and prominent nucleoli and dense orangeophilic cytoplasm.

Table 1: Age distribution table in cases

Age (years)	Normal Pap smears (n=50)	LSIL (Group I) n=25	HSIL (Group II) n=25	SCC (Group III) n=25
20-30	12	-	2	-
31-40	17	12	7	5
41-50	11	8	8	9
51-60	7	4	5	7
61-70	3	1	3	4
Total	50	25	25	25

The nuclear morphometric analysis of normal Pap and abnormal Pap smears were done using Image J software. The results obtained were in pixels. The mean values for the individual cases were calculated and documented in the master chart. The mean values along with standard deviations obtained for all cases of normal Pap smears and abnormal Pap smear groups were considered for the comparison between normal Pap smear and abnormal Pap smear groups.

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, radius and compactness(perimeter2/area) using Image J was carried out and results obtained were 967.98 +/- 386.93, 109.10 +/- 21.74, 39.25 +/- 8.14, 17.21 +/- 3.45 and 12.80 +/- 0.49 respectively.

75 Abnormal Pap smears which were biopsy proven cases comprising of 25 smears each of LSIL, HSIL and SCC were considered for the study. In each of the smears 50 nuclei were assessed and morphometric analysis of nuclear parameters like area, perimeter, maximum diameter, radius and compactness (perimeter2/area) using Image J was carried out and the results obtained for all nuclear parameters as shown in (Table 2).

Table 2: Nuclear morphometric analysis between Normal and Abnormal Pap Smear groups

Nuclear Features		Normal Pap Smears (n=50)	LSIL (n=25)	HSIL (n=25)	SCC (n=25)
Area	Mean	967.98	2640.45	4563.38	4348.84
	SD	386.93	800.70	1595.92	1641.30
	Range	204-3016	284-6044	1060-11292	145-22898
Perimeter	Mean	109.10	184.88	243.66	238.91
	SD	21.74	29.69	46.21	47.97
	Range	21.73-194.77	29.68-311	46.21-431.01	47.96-611.82
Maximum diameter	Mean	39.25	66.01	87.98	87.15
	SD	8.14	11.14	18.00	19.96
	Range	8.13-80.22	11.14-113.84	18.00-151.28	19.95-211.20
Radius	Mean	17.21	28.62	37.52	36.67
	SD	3.45	4.61	6.69	6.30
	Range	3.44-30.98	4.61-43.85	6.68-59.94	6.30-85.37
Compactness	Mean	12.80	13.34	13.42	13.56
	SD	0.49	1.34	1.14	3.99
	Range	0.493-21.86	1.344-24.004	1.139-20.32	3.99-145.01

In the present study we also compared the nuclear parameters between the Abnormal Pap smears using one-way Analysis of Variance (ANOVA) and between the individual Abnormal Pap smear groups, we employed student's' test.

The nuclear area, perimeter, maximum diameter and radius were found to be statistically significant ($p<0.0001$) between the groups of Abnormal Pap smears. (Table 3)

Table 3: Nuclear morphometric analysis between Abnormal Pap smear groups

Nuclear features		LSIL (n=25)	HSIL (n=25)	SCC (n=25)	P value
Area	Mean ± SD	2640.45 ± 800.70	4563.38 ± 1595.92	4348.84 ± 1641.30	<0.0001
	Range	284-6044	1060-11292	145-22898	
Perimeter	Mean ± SD	184.88 ± 29.69	243.66 ± 46.21	238.91 ± 47.97	<0.0001
	Range	29.68-311	46.21-431.01	47.96-611.82	
Maximum diameter	Mean ± SD	66.01 ± 11.14	87.98 ± 18.00	87.15 ± 19.96	<0.0001
	Range	11.14-113.84	18.00-151.28	19.95-211.20	
Radius	Mean ± SD	28.62 ± 4.61	37.52 ± 6.69	36.67 ± 6.30	<0.0001
	Range	4.61-43.85	6.68-59.94	6.30-85.37	
Compactness	Mean ± SD	13.34 ±1.34	13.42 ± 1.14	13.56 ± 3.99	0.952
	Range	1.344-24.004	1.139-20.32	3.99-145.01	

Table 4: P value between Normal And abnormal Pap smears

Nuclear features	p Value		
	Normal & Group I	Normal & Group II	Normal & Group III
Area	<0.0001	<0.0001	<0.0001
Perimeter	<0.0001	<0.0001	<0.0001
Maximum diameter	<0.0001	<0.0001	<0.0001
Radius	<0.0001	<0.0001	<0.0001
Compactness	0.013	0.001	0.186

The results obtained for all nuclear parameters on the Abnormal Pap smears groups were compared with the results obtained by nuclear morphometric analysis performed on Normal Pap smears with the student't' test. The results showed significant difference between Normal Pap smears and Abnormal Pap smear groups with a p- value (< 0.0001) in nuclear parameters area, perimeter, maximum diameter and radius respectively. Also showed a significant difference in compactness between Normal Pap and Group I and II respectively. (Table 4)

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

Nuclear features	p value			
	ANOVA	LSIL - HSIL	LSIL - SCC	HSIL – SCC
Area	<0.0001	<0.0001	<0.0001	0.641
Perimeter	<0.0001	<0.0001	<0.0001	<0.0001
Maximum diameter	<0.0001	<0.0001	<0.0001	0.878
Radius	<0.0001	<0.0001	<0.0001	0.646
Compactness	0.952	0.821	0.795	0.867

Comparison of nuclear nuclear parameters between abnormal Pap smear groups was analyzed with student 't'test. There was significant difference in nuclear area, Perimeter, Maximum diameter and radius between LSIL (Group I) and HSIL (Group II) with a p-value (<0.0001) and LSIL (Group I) and SCC (Group III) with a p-value of (<0.0001) respectively as shown in (Table 5). There was no significant difference in nuclear area (0.641), Maximum diameter (0.878) and radius (0.646) between HSIL (Group II) and SCC with p value of (>0.05). There was no significant difference in compactness between all the three groups (Group I- II =0.821, Group I-III = 0.795, Group II - III = 0.867) with p-value (>0.05). [Table 5]

Table 6: Comparisons between the individual groups among abnormal Pap smears with Bonferroni Multiple Comparisons Test

Dependent variable	Abnormal Pap smear group	Abnormal Pap smear group	p value
Area	HSIL	LSIL	0.0000
		SCC	0.0004
	LSIL	HSIL	0.0000
		SCC	0.0000
Perimeter	HSIL	LSIL	0.0000
		SCC	0.0144
	LSIL	HSIL	0.0000
		SCC	0.0000
Maximum diameter	HSIL	LSIL	0.0000
		SCC	0.6402
	LSIL	HSIL	0.0000
		SCC	0.0000
Radius	HSIL	LSIL	0.0000
		SCC	0.0010
	LSIL	HSIL	0.0000
		SCC	0.0000
Compactness	HSIL	LSIL	1.0000
		SCC	0.4766
	LSIL	HSIL	1.0000
		SCC	0.0964

Comparisons between the individual groups among abnormal Pap smears were also done with post hoc test i.e. Bonferroni Multiple Comparisons Test. There was significant difference in nuclear area, perimeter, maximum diameter and radius between LSIL (Group I) and HSIL (Group II) and LSIL (Group I) and SCC (Group III) with a p-value of (<0.0001). (Table 6)

There was significant difference in nuclear area (0.004), perimeter (0.014), radius (0.001) between HSIL (Group II) and SCC with p value of (<0.05). There was no significant difference in compactness between all the three groups (Group I- II = 1.0, Group I-III = 0.09, Group II -III = 0.47) with p-value (>0.05). (Table 6)

Discussion

"Cervical cancer can have devastating effects with a very high human, social, and economic cost, affecting women in their prime. But this disease should not be a death sentence, even in poor countries," explains Dr Rengaswamy Sankaranarayanan, a lead investigator for an IARC research project with a focus on cervical cancer screening in rural India. "Low-tech and inexpensive screening tools exist and could significantly reduce the burden of cervical cancer deaths right now in less developed countries" [18].

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 [19].

In developed world, large scale screening is done using one of the commercially available automated protocols *viz*, Pap Net, [20] BD Focal Point [21]. The main benefits are increased accuracy, efficiency, and productivity in a cost effective manner. These improvements are beneficial to high-throughput laboratories, which experience increasing volume and workload [21]. However, these are expensive and are not cost effective for low volume work.

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 [17].

The present study deals with the morphometric assessment of Nuclear parameters like Area, Perimeter, Maximum diameter, Radius and Compactness($Perimeter^2$/Area) using Image J software in Normal Pap smears and abnormal Pap smears comprising of LSIL, HSIL and SCC. Normal Pap smears were taken as control for comparison with Abnormal Pap smears.

In the present study, we had confirmed histopathological diagnosis for all selected Abnormal cervical pap smears. LSIL, HSIL and SCC (Ref: fig 2) cases were given Cervical Intraepithelial Neoplasia (CIN I), CIN II/III and squamous cell carcinoma on histopathology respectively.

In the present study the size related parameters (nuclear area, perimeter, maximum diameter and radius) of the nucleus were appropriate parameters to differentiate between normal Pap from abnormal Pap smears. These parameters showed significant differences between normal Pap and abnormal Pap smears which was highly significant with p-value < 0.0001. The nuclear dimensions of cells in abnormal Pap smears were in general significantly much higher than the corresponding nuclear measurements in normal smears (Table 2) which were concurrent with the study conducted by Vijayashree R *et al*. However in their study, they analysed nuclear morphometry in inflammatory and ASCUS smears. Their study showed anisonucleosis, to be a better indicator of neoplasia [16]. In our study we have excluded the inflammatory, ASCUS, ASC-H and AGUS smears.

The other important inference observed that the HSIL and SCC has cells whose nuclei are not only larger but exhibited marked variation in size. This is reflected in high standard deviation 1595.92/1641.30, 46.21/47.97 and 18.00/19.96 for nuclear area, perimeter and maximum diameter respectively between HSIL and SCC groups (table 3). The greater degree of abnormality appeared to be associated with greater anisonucleosis. More than the increase in overall dimensions, high SD (reflecting anisocytosis) appears to be important characteristic of abnormal cells. These findings were also documented in the study conducted by Vijayashree R *et al*. [16].

Table 7: Comparison of measurements of nuclear parameters in normal & abnormal smears in the present study with the study by Vijayashree R *et al*.

Studies	Nuclear parameters	Normal Pap (Mean ± SD)	LSIL (Mean ± SD)	HSIL (Mean ± SD)	SCC (Mean ± SD)
Present study	Area	967.98 ± 386.93	2640.45 ± 800.70	4563.38 ± 1595.92	4348.84 ± 1641.30
	Perimeter	109.10 ± 21.74	184.88 ± 29.69	243.66 ± 46.21	238.91 ± 47.97
	Maximum diameter	39.25 ± 8.14	66.01 ± 11.14	87.98 ± 18.00	87.15 ± 19.96
Vijayashree R *et al*.	Area	710.35 ± 100.24	1784.7 ± 1187.11	2486.16 ± 1229.84	2340.19 ± 1515.57
	Perimeter	105.89 ± 8.54	167.62 ± 56.67	194.47 ± 49.42	191.15 ± 62.44
	Feret diameter	35.43 ± 2.74	58.34 ± 18.09	67.7 ± 16.49	66.35 ± 20.3

In the present study we also studied the size related parameters (nuclear area, perimeter, and diameter) of the nucleus were appropriate parameters to differentiate between premalignant (LSIL and HSIL) from malignant (SCC) cervical smears. These parameters showed significant differences between LSIL, HSIL and squamous cell carcinoma which was highly significant with p-value < 0.0001(Tables 4 and 5) that showed similar results when compared with the study conducted by Divyarani *et al*. [17] (Table 8)

Table 8: Comparison of ANOVA p values in nuclear morphometric analysis between the groups in Abnormal Pap smears between the present study and study by Divyarani *et al*.

Nuclear parameters	ANOVA p value	
	Present study	Divyarani *et al*.
Nuclear Area	<0.0001	<0.0001
Perimeter	<0.0001	<0.0001
Maximum diameter	<0.0001	<0.0001
Radius	<0.0001	<0.0001
Compactness	0.952	>0.05

Many studies were conducted in analyzing the nuclear features by morphometry in other organs like breast, exfoliated buccal mucosal cells, squamous neoplasms, colon and thyroid.

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 [22].

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 [23].

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 [15].

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 [24].

Ikeguchi M *et al*., studied morphometric nuclear features (nuclear area, perimeter, and shape) in 343 patients with colorectal carcinoma and in 57 patients with colorectal adenoma and found that the mean nuclear area enlarged from normal colorectal mucosa to adenoma and carcinoma [25].

Deka L *et al*., studied nuclear morphometry and texture analysis on cytological smears of thyroid neoplasms comprising of 20 colloid goiter, 20 follicular neoplasms and 10 papillary carcinoma and found that cases of papillary carcinoma showed the highest perimeter, area, radius and elongation factor while roundness factor was the lowest in papillary carcinoma among the three groups [26].

Yashaswini *et al*. studied Bethesda and thyroid morphometry and found that Minimal nuclear diameter, maximal nuclear diameter, nuclear perimeter, and nuclear area were higher in malignant group compared to nonneoplastic and benign group [27]

The results of nuclear morphometry using various parameters like area, perimeter, diameter, radius and compactness can be made even more précised 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 [28].

Nuclear morphometry can also be utilised as a diagnostic tool especially in gray zones [23, 26] on cervical smears, especially by ASCUS or AGUS which are encountered during reporting of Pap smears [17].

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, diameter and radius to be of more significance in differentiating the premalignant and malignant lesions. The studies by Yashaswini *et al*. [27] and Narasimha A *et al*. [29] also showed compactness to be a significant nuclear parameter between the benign and malignant conditions in their study (table 13). In the present study nuclear compactness was not found be a significant parameter to differentiate between abnormal Pap smear groups.

The present study showed there was a gradual increase in nuclear area and perimeter in carcinoma when compared to premalignant lesions (LSIL and HSIL). The nuclear morphometric parameters which could significantly differentiate between LSIL and HSIL were nuclear area, perimeter, diameter and radius. These four parameters were useful to differentiate between LSIL and SCC which was statistically significant. Thus nuclear area, perimeter and diameter were highly significant in differentiating premalignant from malignant cervical smears with p-value of < 0.0001. Compactness was not statistically significant in differentiating between all the three groups with p-value

>0.05 and thus there was no discrepancy noted in compactness of cell nuclei in any of the three groups which was in concordance with Divyarani *et al*. study [17].

Conclusion

The present study showed that the nuclear morphometric parameters related to nuclear size like area, perimeter, maximum diameter and radius were significantly larger in abnormal Pap smears group than the Normal Pap smears group. The nuclear morphometric parameters like area, perimeter and maximum diameter were also found to be significant between each category i.e., LSIL (Group I), HSIL (Group II) and SCC (Group III) respectively of the abnormal Pap smears. In the present study it was found that the mean values for nuclear area, perimeter and maximum diameter of SCC smears was lower than the corresponding nuclear parameters found in HSIL smear groups. But the higher standard deviation values showed significance in SCC smear group indicating a greater variation in cell size (anisonucleosis).Hence we can conclude that in Pap smears, anisonucleosis detected by nuclear morphometry is a better marker of neoplasia than changes in the dimensions of the nuclei.

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