IJPRP

Indian Journal of Pathology: Research and Practice 8(3) May - June 2019
DOI: http://dx.doi.org/10.21088/ijprp.2278.148X.8319.10

Original Research Article

Significance of Nuclear Morphometry in Evaluation of Squamous Intraepithelial Lesions in Vaginal Cytology: A Case Series Study

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How to cite this article:

Vedamoorthy Ravishankar, B. Pushpa. Significance of Nuclear Morphometry in Evaluation of Squamous Intraepithelial Lesions in Vaginal Cytology: A Case Series Study. Indian J Pathol Res Pract. 2019;8(3):317-323.

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Received on 03.03.2019,

Accepted on 27.03.2019

Abstract

Context: Cervical cancer is the second leading malignancy in the women occurring worldwide. Researches based on nuclear morphometry on cervicovaginal smears are very much sparse; hence we have taken up this study. *Aims:* To valuate the role of nuclear morphometric parameters (nuclear radius and area) in differentiation of premalignant lesions of cervix. *Settings and Design:* Retrospective analysis of nuclear morphometric parameters in cervical smears of 40 cases. *Methods and Material:* Retrospective analysis of nuclear morphometric parameters in 40 cervical smears (20- LSIL and 20- HSIL) done at Government Kilpauk Medical College. *Statistical analysis used:* Independent sample t test was used for comparing radius and nuclear area in LSIL and HSIL lesions. *Results:* There was an appreciable difference in the area of nucleus among the premalignant lesions. The mean nuclear area in LSIL was 109 ± 7.446 um^2 and HSIL was 132.48 ± 11.388 um^2. Radius of the nucleus also showed appreciable difference in LSIL and HSIL cases included in our study. The mean nuclear radius in LSIL lesions was 3.78 ± 0.25 um and in HSIL lesions was 4.37 ± 0.34 um. *Conclusions:* Nuclear morphometry is henceforth an useful and dependable investigative tool in the differentiation of premalignant cervical lesions.

Keywords: Nuclear morphometry; LSIL,HSIL.

Introduction

Cervical malignancies are the second leading malignancies to be prevalent among the women worldwide [1]. Cervical malignancies are more prevalently seen among the indian women and one among the leading causes of cancer related mortality in women. Cervical cancer arises from a precursor lesions, known as intraepithelial lesions which develops from a normal cells after several years. To make a diagnosis of a premalignant lesion, it is necessary for a Cytologist to detect the

atypical cells in a Papanicolou stain applied cervical smears [2].

A marked reduction in incidence and malignancy related mortality has been brought down by Pap smear screening which is primary modality of screening in cervical cancers. Cervical cancer screening helps in early detection and reduction of mortality relating to cervical malignancies. As a result, Cytological evaluation of these atypical cells is of paramount importance in early detection of dysplasia or pre-invasive cervical carcinomas.

However, errors resulting from sampling techniques, screening and interpretation, disclosed some disagreeable results [4]. The precision of diagnosis done on pap smears principally depends on the morphology of atypical cells. Cytological changes produced by acquired infections, drugs intakes, hormonal alterations may closely simulate the abnormal atypical cells [2]. In these circumstances, Colposcopy directed biopsy is needed for conclusive diagnosis [5]. When atypical cells are evident on a pap smear, the appreciation of premalignant or malignant lesion is highly under subjective judgement and variability.

In order to reduce the false negativity in interpretation, Cowpe et al. [6] Goldsby et al. [7], have recommended the usage of quantitative parameters, that depend on the analysis of variables, such as area of the nucleus, area of the cytoplasm, and ratio of nucleus : cytoplasm [8]. This quantitative analysis of nuclear and cytoplasmic parameters enhanced the precision of diagnosis of pre-malignant lesions. Studies on nuclear morphometric analysis of cervical smears are very much low. Henceforth, we have taken up this study.

Materials and Methods

Our study was centered upon retrospective analysis of 40 cervical pap smears received at our department of pathology, Government Kilpauk Medical College, Chennai. Cases included in our study population had confirmation with histopathological diagnosis.

Bethesda system was applied to classify cervical smears into 2 categories.

LSIL (Low grade squamous intraepithelial lesion included 20 cases)

HSIL (High grade squamous intraepithelial lesion included 20 cases)

Image Analysis was done by using a microscope with a 2.5x eye piece and an objective of 40x magnification to select a field for analysis. A digital image of the field of resolution 640 x 480 pixels was captured by a camera on the microscope and frame card connected to computer. Images captured were saved in the computer's memory space. Around 20 nuclei/case were studied by using Scopeimage 9.0 morphometric software for processing image and analysis.

Scopeimage 9.0 morphometric software is especially designed for digital microscope; it has friendly operation interface, stable performance and powerful function, very easy to operate. And can be widely used in various optical micro fields, such as teaching, researching, electronic checking and so on. It is image-processing software professionally designed for digital microscope. It allows you to view, capture, edit, record, zoom, measure, and process microscope images. The measurements were done on the cell images in a reproducible way, and stored for future analysis.

Nuclear parameters analysed:

Radius measured by taking the mean of the length of line drawn radially from the center of the nucleus to a point on the membrane of nucleus.

Area of the nucleus was measured the area within the encircled nuclear membrane.

Results

Our study analysis had included 40 cases with ages ranged from 21-70 years. The age distribution of the cases are shown. The study population was divided into 2 groups based on atypical cytological features:

LSIL (Low grade squamous intraepithelial lesion had 20 cases)

HSIL (High grade squamous intraepithelial lesion had 20 cases)

Atypical cell features of LSIL (Low grade squamous intraepithelial lesion):

Single or sheets of superficial and intermediate cells, Abundant cytoplasm. Nucleus–Enlarged nucleus: Nucleus occupy more than one-third of the cytoplasmic area (3 to 5 times of a normal intermediate cell nucleus), Mild hyperchromasia, Irregular margin, Evenly distributed fine granular chromatin–Nucleoli absent, Low nucleo-cytoplasmic (N/C) ratio [10] (Fig. 1).

Atypical cell features of HSIL (High grade squamous intraepithelial lesion) – Single or sheets of cells, parabasal or basal type of cell,

cytoplasm is dense or lacy and orangeophilic in keratinized variant. Nucleus -Three times size of intermediate cell nuclei, Moderately pleomorphic, Hyperchromatic. Marked irregular nuclear membrane, Coarse chromatin, Prominent nucleoli. Nuclear - cytoplasmic ratio is high [10]. (Fig. 2).

The maximal age occurrence of LSIL cases included in our study were of the ages ranging from thirty one to forty years old, HSIL were in between forty one - fifty years old. (Table 1).

Nuclear morphometric variables were studied using Scopeimage 9.0 software. The observed results of our study were tabulated in (Table 2).

Area of the atypical cell nucleus had an appreciable difference between LSIL and HSIL populations. The mean nuclear area in LSIL was 109 ± 7.446 um^2 and HSIL was 132.48 ± 11.388 um^2. (Table 2)

Radius of the atypical cell nucleus also varied significantly between LSIL and HSIL populations included in our study. The mean nuclear radius in LSIL lesions was 3.78 ± 0.25 um and in HSIL lesions was 4.37 ± 0.34 um (Table 2).

The results of our study were found to be in correlation with previous study on Evaluation of Pre-Malignant and Malignant Lesions in Cervico Vaginal (PAP) Smears by Nuclear Morphometry by Divya Rani M. N et al. whose findings were as follows [21]:

Nuclear area in LSIL lesions were 109.54 ± 11.13 um^2 and in HSIL lesions were 132.7 ± 17.31 um^2.

Nuclear radius in LSIL lesions were 3.96 ± 0.18 um and in HSIL lesions were 4.31 ± 0.29 um.

The mean nuclear area, nuclear radius were the useful parameters in differentiation of LSIL and HSIL lesions. By using Independent sample T test, nuclear radius and area were found to be statistically significant ($p<0.001$) (Table 3). The graphical representation of the observed results are shown (Table 4 and 5).

Table 1: Age Distributuon of Cases

Age distribution (Years)	Low grade squamous intraepithelial lesion(LSIL)	High grade squamous intraepithelial lesion (HSIL)
21-30	3 (15%)	0 (0%)
31-40	8 (40%)	2 (10%)
41-50	5 (25%)	11 (55%)
51-60	2 (10%)	5 (25%)
61-70	2 (10%)	2 (10%)
Total	20	20

Table 2: Observations and Results

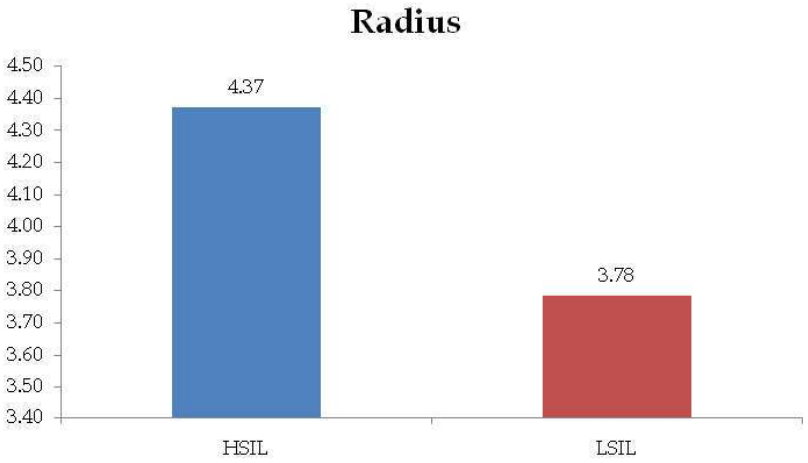
Nuclear features	Normal intermediate cell nuclei	Low grade squamous intraepithelial lesion (n=20)(LSIL)	High grade squamous intraepithelial lesion (n=20)(HSIL)
Nuclear area	35-50 um^2	109 ± 7.446 um^2 (101.55 -116.44)	132.48 ± 11.388 um^2 (121.09 – 143.868)
Nuclear radius	3.25 – 3.5 um	3.78 ± 0.25 um (3.53 – 4.03)	4.37 ± 0.34 um (4.03 – 4.71)

Abbreviations: um -micrometre.

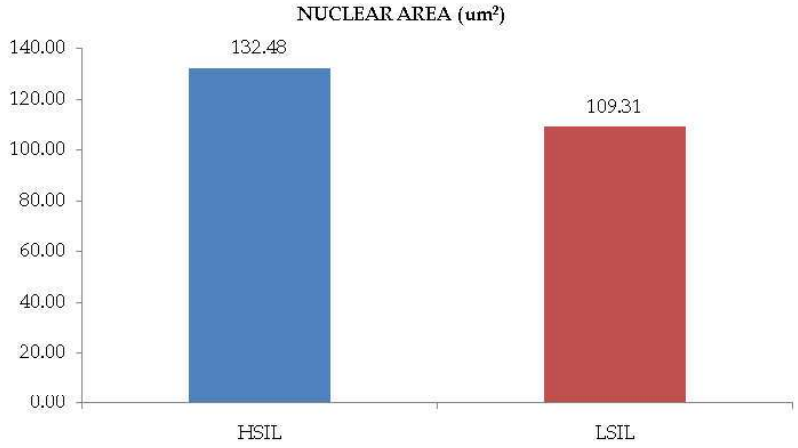
Table 3: Statistical analysis of the observed results.

Independent Samples Test						
	Group	N	Mean	Std. Deviation	Std. Error Mean	t value
Radius	HSIL	20	4.3725	.17483	.03909	12.103**
	LSIL	20	3.7840	.12931	.02891	
Nuclear Area (um^2)	HSIL	20	132.4762	11.68387	2.61259	7.420**
	LSIL	20	109.3129	7.64235	1.70888	

**p<0.001 (highly significant)



Graph 1: Graphical representation of observed results (nuclear radius)



Graph 2: Graphical representation of observed results (nuclear area)

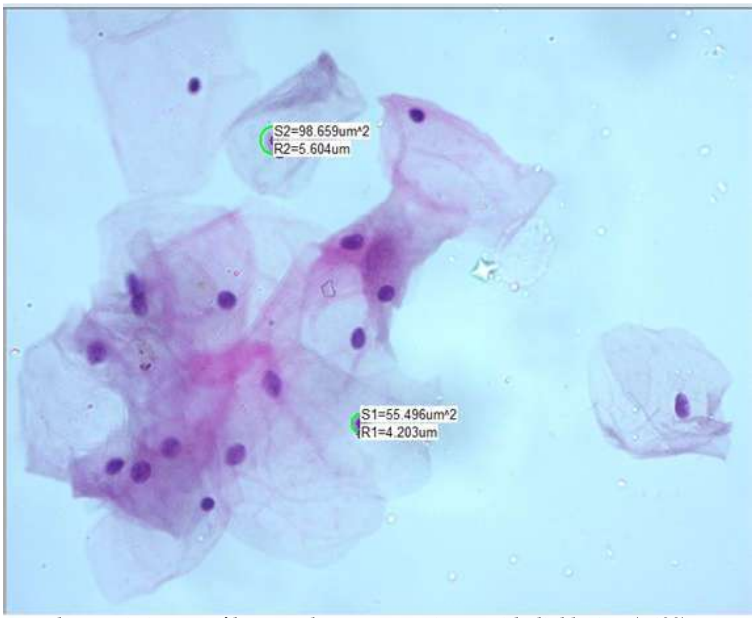


Fig. 1: Nuclear morphometric image of low grade squamous intraepithelial lesion (x400). (Hematoxylin and eosin)

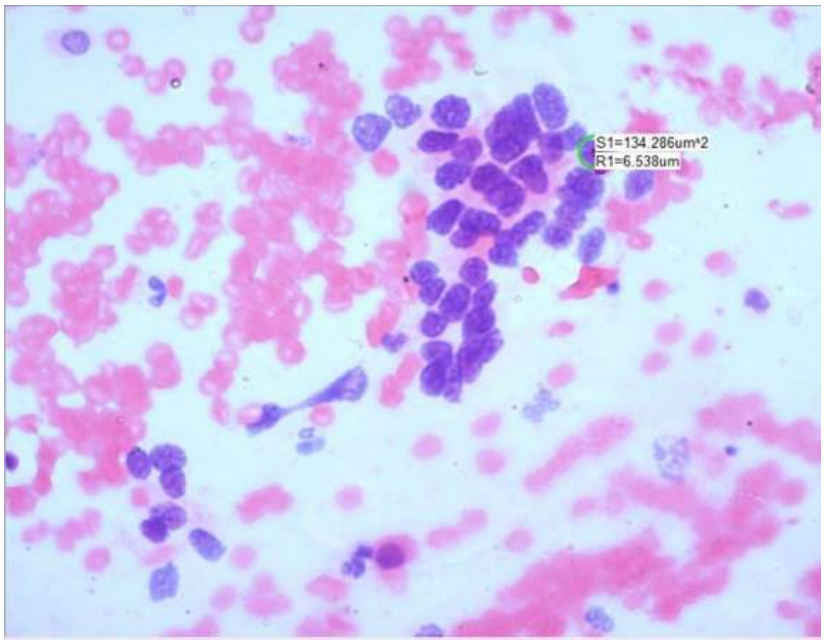


Fig. 2: (A) – Nuclear morphometric image of High grade squamous intraepithelial lesion (x400) (Hematoxylin and eosin)

Discussion

Carcinoma cervix has the second most global prevalence datas for cancers amongst women [11]. An estimated detection for cervical cancers were 3,71,000 new cases and about 1,90,000 deaths annually. Developing country like India has a share of about 80% of these new cases [12]. Papanicoloau cervicovaginal smear screening is a vital screening test of a woman's essential health check- up. It is a widely used screening test in detection of atypical cells in premalignant lesions of cervix, as well as in malignancy. Both are treatable successfully if diagnosis is made at a much earlier stages. Regular cervical malignancy screening has been proven greatly to bring down the number of new incident cases diagnosed annually and mortalities from malignancy [13].

Care need to be exercised while reporting Pap smears. In spite of well- developed screening programs in United States, nearly 50% of the cervical malignancies are diagnosed in well advanced stages. In a developing country like India, the disease is usually well spread at the time of initial presentations leading to a high mortality rate among women [1].

Hence, our study was aimed at assessing the role of nuclear morphometric variables in the recognition of true positives and true negatives for differentiation of pre-malignant lesions. Criteria for recognising normal cells from atypical cells were based on changes in size of the nucleus, irregularity in the nuclear contour that are assessed in cervical smears based on observer's judgement [4]. Contrastingly, in computerised morphometry, these subjective variables are measured as quantifiable parameters [4].

Morphometric features of squamous cells are as follows-

- Basal cells with nuclear diameter of 7-9 um and area of 50 um^2 are round to oval cells with scanty cytoplasm.
- Parabasal cells are with round to oval nucleus and granular chromatin has nuclear diameter of 5-7 um and area of 35 um^2.
- Intermediate cells are polygonal cells with abundant cytoplasm and has round nuclei with granular chromatin, has nuclear diameter of 10-12 um and area 36 um^2.
- Superficial cells are polyhedral cells with abundant cytoplasm with round pyknotic nuclei having a diameter of 5 – 7 um and 15 um^2 nuclear area.

In our study, we had confirmed histopathological diagnosis for all the included study populations. LSIL cases were reported as Mild dysplasia, HSIL were reported as moderate to severe dysplasia on histopathology. The mean area of nucleus, radius of nucleus were useful parameters in differentiating the pre-malignant lesions. These parameters had a significant difference in LSIL and HSIL lesions. By

using Independent sample T test, we found nuclear area and radius to be statistically significant in differentiating HSIL and LSIL lesions. Findings of our study were similar to the nuclear morphometric study on cervical cytology by Divya rani et al. [21].

Some of the studies on breast carcinomas have also analysed longest axis and shortest axis as nuclear morphometric variables, but among the many nuclear variables, nuclear area and radius were statistically significant [17-18].

In addition to the basic nuclear morphometric variables, Murata et al., analysed 27 morphometric variables confined to nucleus which were grouped into more agreeable cytological features for better understanding and correlation of computed morphometric features [19].

In a study by Huang et al., done on cervicovaginal smears by computer based Image Analysis System and Support Vector Machine showed that in dysplastic atypical cells, the morphometric variables like perimeter of nucleus, area of nucleus, N/C ratio were found to be significant. The statistics proved that dysplastic cells had a larger nuclear area and radius, higher nuclear proportion (i.e. N/C ratio) compared to normal cells [4].

Prasad et al., studied on buccal mucosal cells which had exfoliated in diabetes patients by cytomorphometric analytic study and showed that there was an appreciable increase in diameter of the nucleus in diabetics as compared to controls [20].

Conclusion

Nuclear morphometry is henceforth an useful objective tool in the discrimination of premalignant lesions. It is also useful in diagnostic grey zone areas encountered in cervical smears especially ASCUS or AGUS. Hence, the combination of clinical findings, cytomorphologic study and morphometric variables can be applied in the improvisation of the diagnostic power of cervical malignancy screening modalities.

Acknowledgement: Nil

Conflict of Interest: There was no Conflict of Interest.

Appendixes (if necessary): Nil

Abbreviations (if necessary):

LSIL- Low grade squamous intraepithelial lesion

HSIL- High grade squamous intraepithelial lesion

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