

ROLE OF MORPHOMETRY IN DIAGNOSING PREMALIGNANT AND MALIGNANT LESIONS IN LIQUID BASED CYTOLOGY CERVICAL SMEAR(PAP SMEAR)

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Abstract:

Background: Cervical cancer is the second most common cancer in India. The pathogenesis from low grade CIN to cervical cancer takes from 10 to 20 years, during which timely screening for premalignant lesions and early treatment is highly effective in preventing overt disease.

Aims: To study the morphometric parameters of the LBC Pap smear and correlate with morphometric parameters, exploring its possible role to improve the sensitivity and specificity for detection of premalignant and malignant conditions.

Design: Prospective analytical study.

Methods and Material: The morphometric analysis was conducted on 200 LBC cervical samples which included normal and abnormal case findings. 20 intermediate squamous cells per slide were evaluated using Nikon instrument software. Cases screened and reported as squamous cell abnormality, cases showing reactive cellular changes and cases having normal cytology were included. The parameters evaluated were cell area, cell perimeter, nuclear area, nuclear diameter, nuclear perimeter and N:C ratio. The LBC technique used was Surepath test.

Statistical analysis used: The parameters between the normal and abnormal Pap smear groups were compared using UNPAIRED t-test. Software used was SPSS Version21.

Results: It was observed that there was increase in N:C ratio, nuclear area, nuclear diameter, nuclear perimeter from normal to squamous epithelial lesions. Mean cell area and mean cell perimeter was more in normal cells compared to abnormal squamous epithelial lesion.

Conclusion: Morphometry can be used as a diagnostic tool in differentiating between premalignant and malignant lesions of cervix.

Keywords: Morphometry, LBC, squamous epithelial lesions.

Introduction: Cervical cancer is the second most prevalent cancer among women in less developed countries and the fourth most common cancer overall in women.^[1] Cervical cancer begins with a precancerous stage, known as dysplasia which takes many years to develop from a normal cell.

The primary cause of cervical cancer is human papillomavirus (HPV). The most common oncogenic type is HPV 16. Cervical cancer has a high 5-year survival rate of up to 92% and is treatable if found early. The development of cervicovaginal cytology as a method to identify uterine cervix precancerous lesions has been a significant step in the research of uterine cervix cancer. When abnormal cells are found on a Pap smear, cancer and precancerous conditions might be diagnosed.^[2]

Incidence and death of cervical cancer have been significantly reduced by 85% as a result of pap smear screening, which is still the primary method for identifying premalignant lesions and cervix carcinoma.^[3] Liquid-based cytology (LBC) has been introduced into cytopathology, primarily in the field of cervical cytology, with the goal of enhancing diagnostic accuracy. The morphological criteria are mostly based on descriptive nuclear changes. By using objective methods like morphometry with computer-assisted image analysis, it is possible to avoid false interpretations, identify borderline cases, and treat patients more effectively and quickly. Morphometry is the quantitative description of structural geometric characteristics such as cells, nuclei, or nucleoli. The study of nuclear morphometry in identifying benign from malignant lesions based on their cellular, nuclear properties is one of the most significant tasks of morphometry in pathology.

Materials and Methods: The study was conducted on 200 LBC cervical samples which included normal and abnormal case findings, 20 intermediate squamous cells per slide were evaluated using Nikon Instruments Software (NIS-Elements Documentation(D) 5.01.00).

Cases screened and reported as squamous cell abnormality, cases showing reactive cellular changes and cases having normal cytology were included.

Unsatisfactory samples, patients on intravaginal drugs and already diagnosed SCC cases on radiotherapy were excluded.

The parameters evaluated were cell area, cell perimeter, nuclear area, nuclear diameter, nuclear perimeter and N:C ratio.

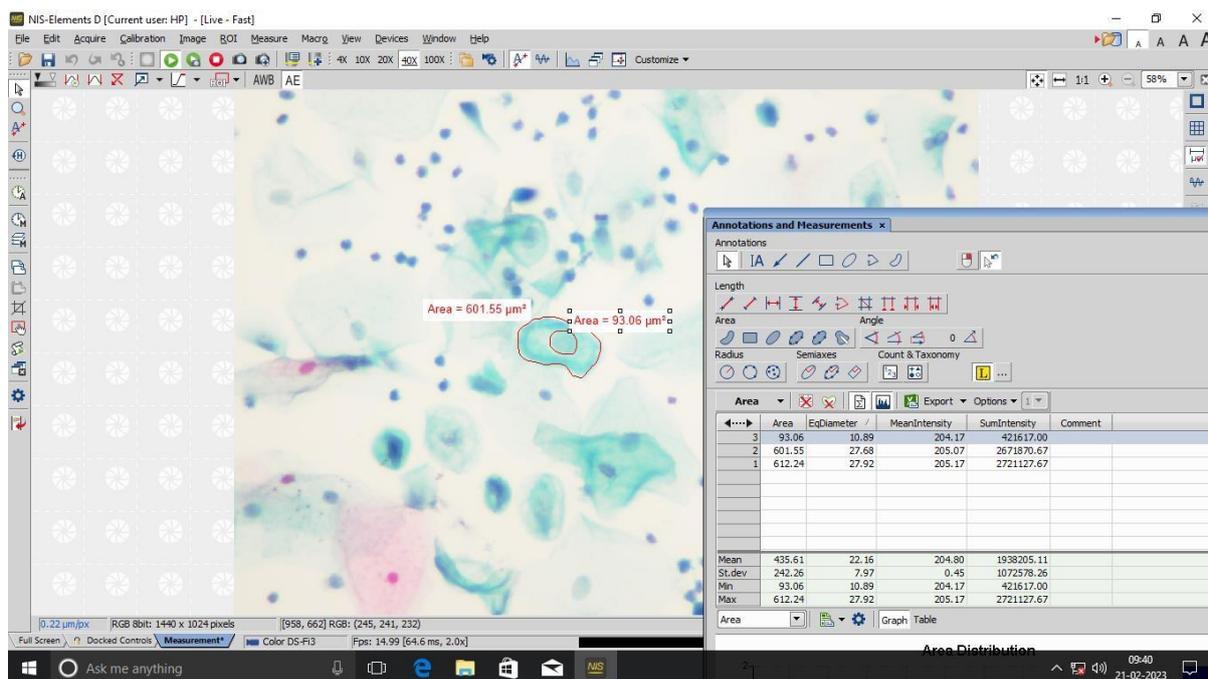
1. Cell area: area within the outlined cell perimeter.
2. Cell perimeter: length around the cell border.
3. Nuclear area: area within the outlined nuclear perimeter
4. Nuclear perimeter: length around nuclear border
5. Nuclear diameter: The diameter within the same area as the measured nucleus.
6. N:C Ratio: Ratio of nuclear area and cytoplasmic area.

The LBC slides were prepared using BD Surepath method.

STATISTICAL ANALYSIS

The results obtained by computerized Cyto-morphometry were compared between the normal Pap smears and abnormal Pap smears groups. The most distinctive morphometric features among the various morphometric features were analyzed. Data between normal and Epithelial cell abnormalities was analyzed by analysis of variance test (ANOVA), and individual analysis between groups was done by UNPAIRED T test. Software used was SPSS

Version 21. A p-value < 0.05 was considered as statistically significant



[Table/Fig-1]: Morphometric image analysis representation using NIS-Elements software.

RESULTS:

Maximum number of cases belonged to 31-40 yrs age group(55%) with normal group cases ranging from 20 -40 years , reactive cases ranged from 20 to 50 years, and age group for epithelial cell abnormality cases ranged from 20 to 70 years. (Table 1)

It was observed there was gradual decrease in mean cell area, mean cell perimeter from normal group to SCC. ASC-H had the lowest mean cell area and perimeter.

There was gradual increase in mean nuclear area, mean nuclear perimeter, mean nuclear diameter from normal group to SCC. However, mean nuclear area, mean nuclear diameter in ASC-H was lower than ASCUS and LSIL.

Mean N:C ratio showed gradual increase from normal group to SCC. (Table 2)

On comparing the parameters between epithelial cell abnormalities, cell perimeter was not found to be statistically significant (Table 3)

Age (yrs)	NORMAL		REACTIVE		ASCUS		LSIL		ASC-H		HSIL		SCC		Total	
	n*	%	n	%	n	%	n	%	n	%	n	%	N	%	n	%
20-30	27	36.00	30	36.59	5	25.00	3	37.50	0	0.00	0	0.00	0	0.00	65	32.50
31-40	42	56.00	49	59.76	14	70.00	5	62.50	1	25.00	0	0.00	0	0.00	111	55.50
41-50	6	8.00	3	3.66	1	5.00	0	0.00	3	75.00	4	66.67	0	0.00	17	8.50
51-60	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	2	33.33	3	60.00	5	2.50
61-70	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	2	40.00	2	1.00
Total	75	100.00	82	100.00	20	100.00	8	100.00	4	100.00	6	100.00	5	100.00	200	100.00

*n=number of cases

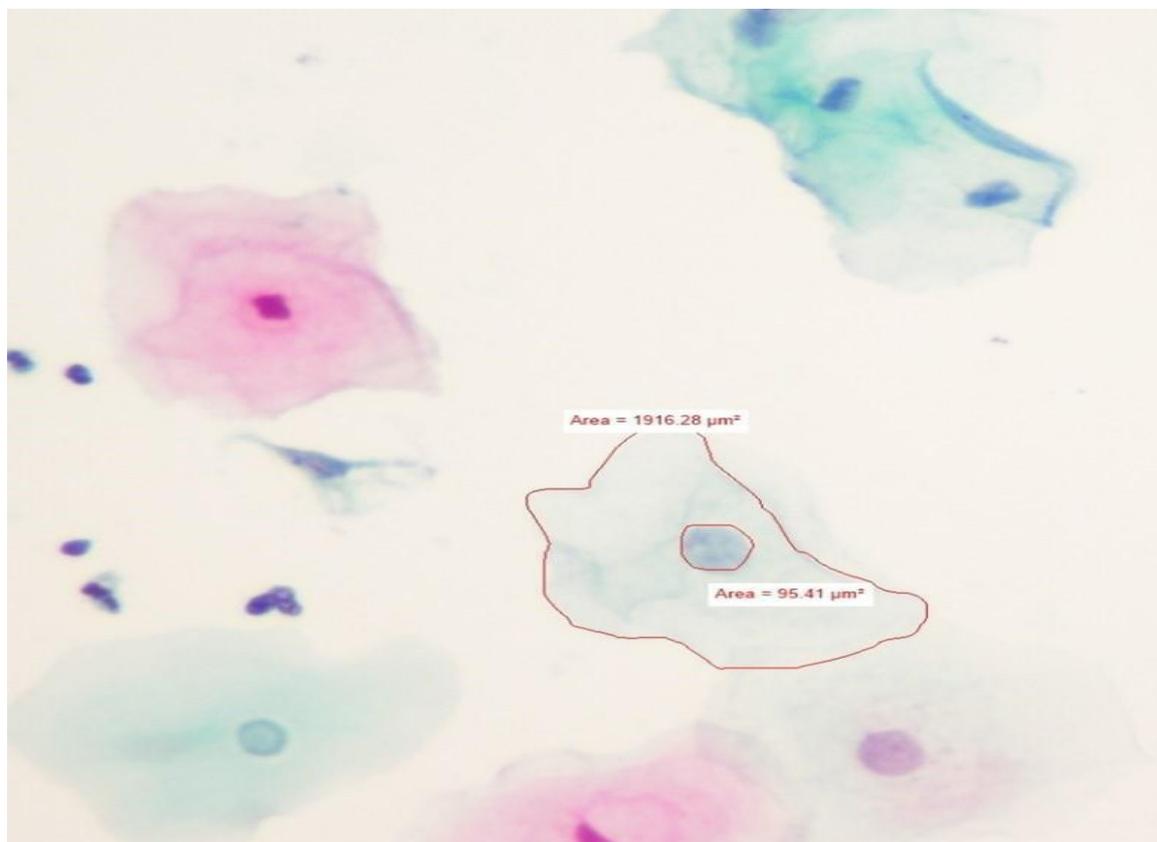
[Table/Fig-2]: Showing age wise distribution

Features	Normal		Reactive		ASCUS		LSIL		ASC-H		HSIL		SCC		F-ratio	p value
	Mean	±SD	Mean	±SD	Mean	±SD	Mean	±SD	Mean	±SD	Mean	±SD	Mean	±SD		
Cell Area	2061.80	263.96	1514.40	304.48	1291.90	256.83	1084.20	298.81	603.46	75.87	698.06	199.94	674.09	93.21	73.281	<0.001
Cell Perimeter	502.78	32.23	421.27	54.44	404.73	44.24	483.09	390.41	311.52	45.62	311.97	21.84	336.15	36.07	12.942	<0.001
Nuclear Area	40.33	2.91	79.27	9.91	100.12	11.81	104.14	13.89	82.91	9.42	112.49	21.58	152.81	24.58	267.2	<0.001
Nuclear Perimeter	67.72	2.85	91.48	9.54	105.21	5.67	108.97	8.07	105.75	11.20	111.01	11.53	132.52	7.34	172.44	<0.001
Nuclear Diameter	7.16	0.26	10.02	0.63	11.19	0.59	11.58	0.69	10.31	0.46	12.27	1.10	14.12	0.97	378.4	<0.001
Nuclear: Cytoplasmic Ratio	0.020	0.003	0.054	0.012	0.083	0.019	0.102	0.030	0.130	0.008	0.160	0.028	0.222	0.033	345.58	<0.001

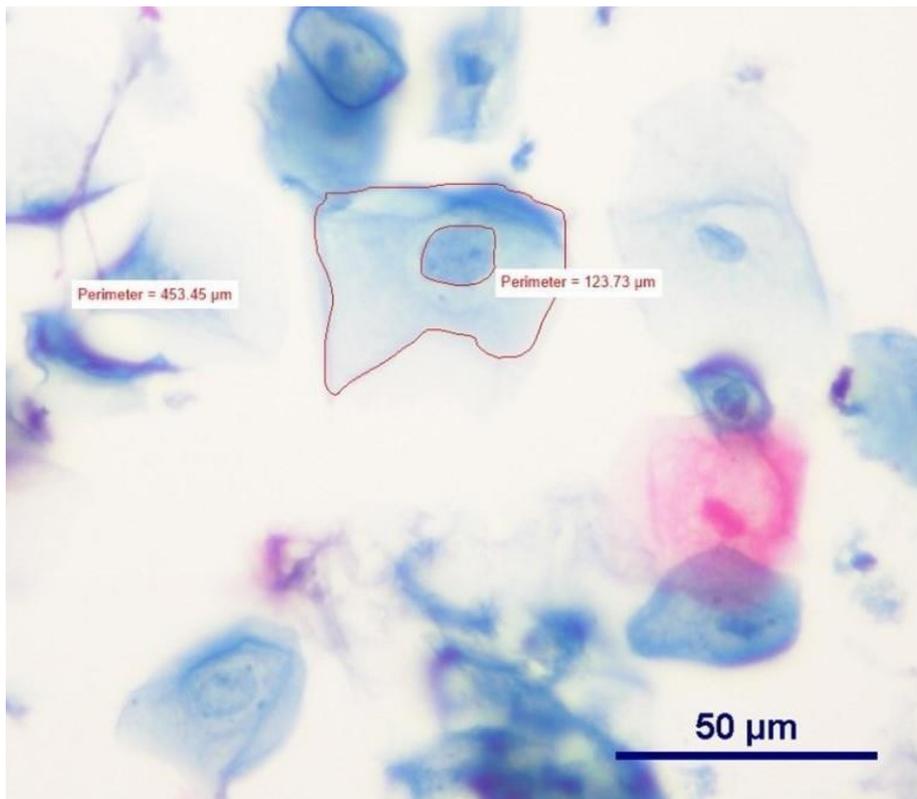
[Table/ Fig-3]: Morphometric analysis between the groups.

Features	ASCUS		LSIL		ASC-H		HSIL		SCC		F-ratio	P-value
	Mean	±SD	Mean	±SD	Mean	±SD	Mean	±SD	Mean	±SD		
cell area	1291.90	256.83	1084.20	298.81	603.46	75.87	698.06	199.94	674.09	93.21	15.238	<0.001
cell perimeter	404.73	44.24	483.09	390.41	311.52	45.62	311.97	21.84	336.15	36.07	1.269	0.299
nuclear area	100.12	11.81	104.14	13.89	82.91	9.42	112.49	21.58	152.81	24.58	14.764	<0.001
nuclear perimeter	105.21	5.67	108.97	8.07	105.75	11.20	111.01	11.53	132.52	7.34	12.607	<0.001
nuclear diameter	11.19	0.59	11.58	0.69	10.31	0.46	12.27	1.10	14.12	0.97	20.689	<0.001
nuclear: cytoplasm	0.083	0.019	0.102	0.030	0.130	0.008	0.160	0.028	0.222	0.033	40.825	<0.001

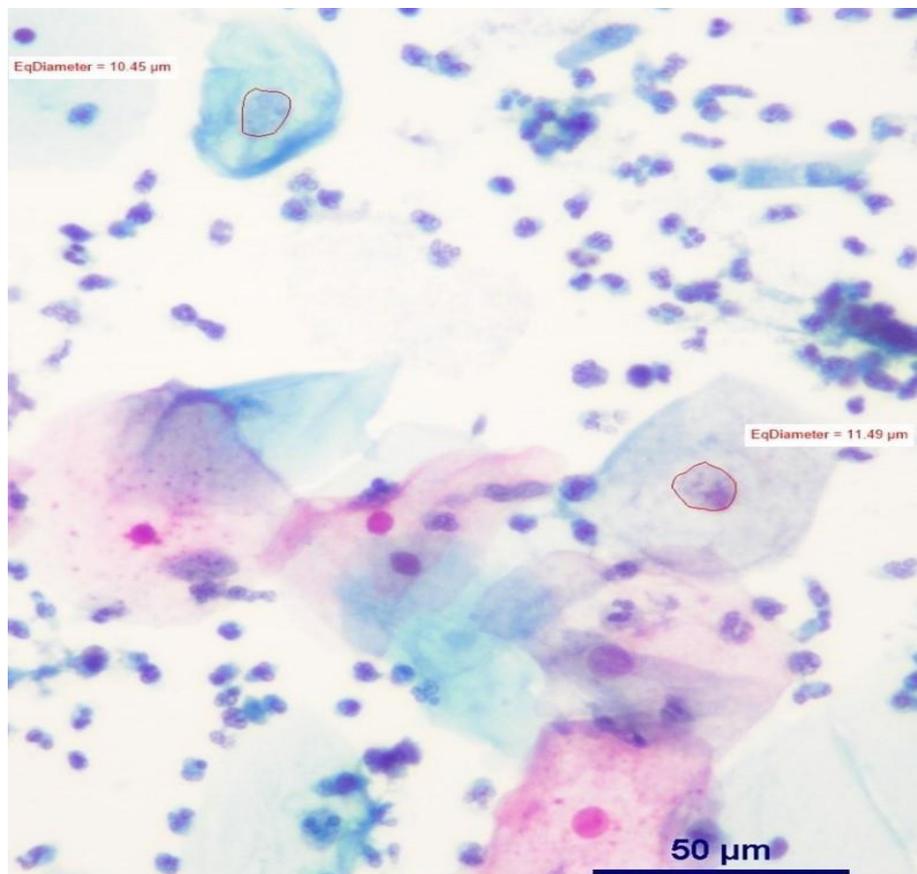
[Table/ Fig- 4]: Morphometric analysis between epithelial cell abnormalities.



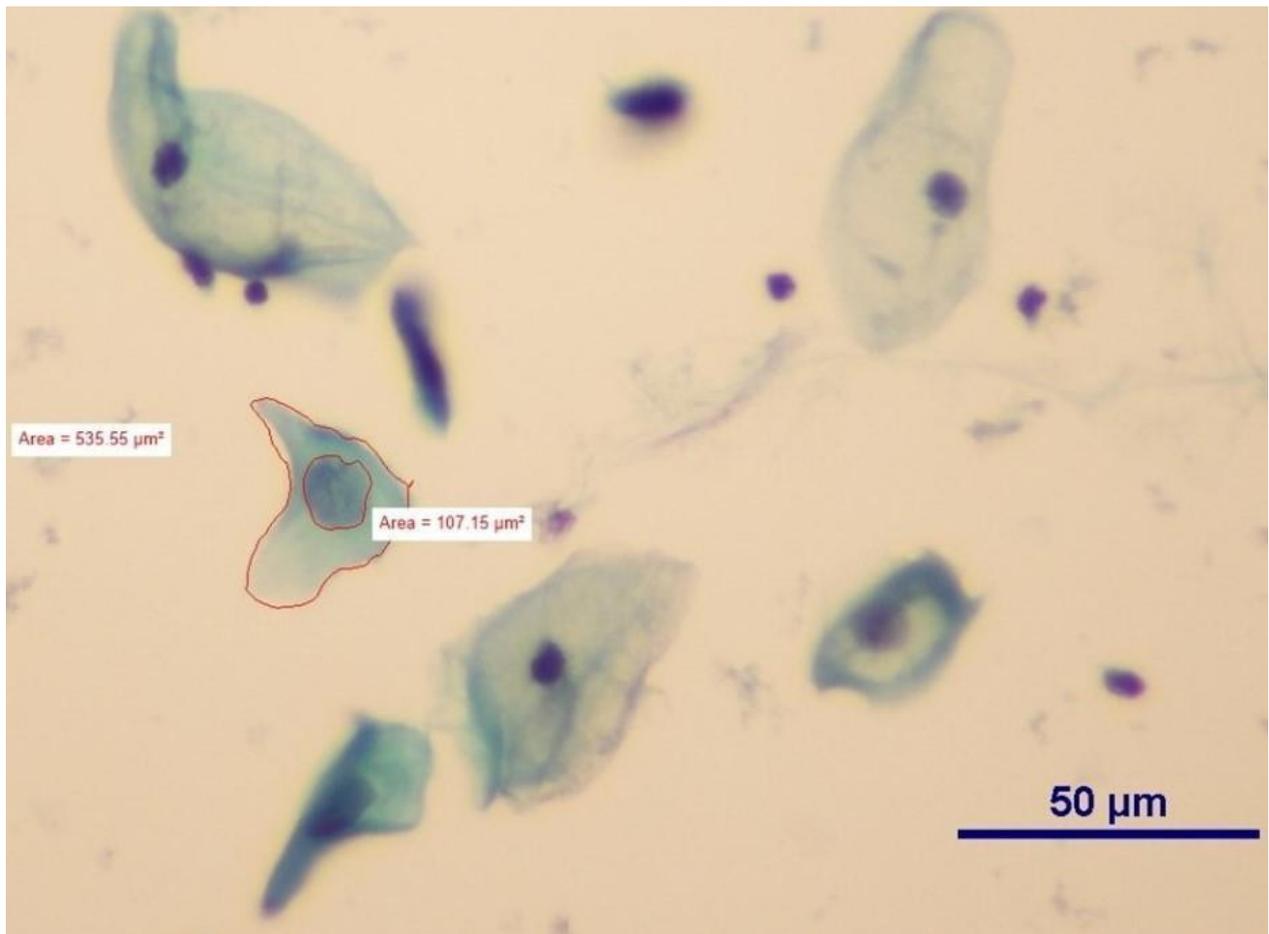
[Table/ Fig- 5]: Measuring cell and nuclear area of a reactive case (BD SUREPATH 40X)



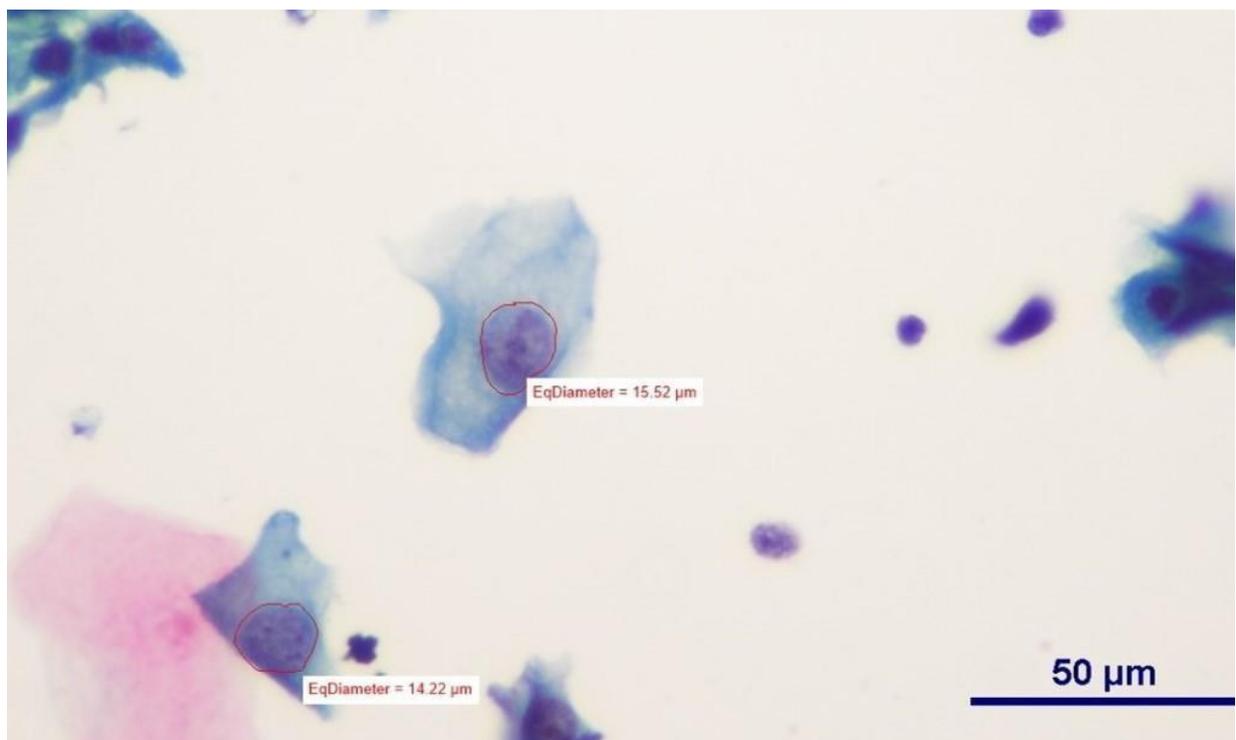
[Table/ Fig- 6]: Measuring cell and nuclear perimeter of case diagnosed as ASCUS (BD SUREPATH 40X)



[Table/ Fig- 7]: Measurement of nuclear diameter of a case diagnosed as LSIL (BD SUREPATH 40X)



[Table/ Fig- 8]: Measurement of cell and nuclear area of a case diagnosed as HSIL (BD SUREPATH 40X)



[Table/ Fig- 9]: Measurement of nuclear diameter of a case diagnosed as SCC. (BD SUREPATH 40X)

DISCUSSION:

In the present study, the size related parameters (cell area, cell perimeter, nuclear area, perimeter, diameter, N:C ratio) were appropriate parameters to differentiate between normal from reactive to ECA (Epithelial Cell Abnormality) in cervical smears.

In our study, cell perimeter was not statistically significant in differentiating between ASCUS, ASC-H, LSIL, HSIL, SCC with p-value = 0.299.

In the present study it was found that there was gradual decrease in cell area from normal to dysplastic and SCC with ASC-H having the smallest mean cell area. Our findings are consistent with other studies in literature^[4,5,6].

Findings from the current study showed that there was decrease in cell perimeter from normal to dysplastic to SCC with ASC-H having the lowest mean cell perimeter, which is in correlation with study conducted at Subharti medical college, Meerut, U.P.^[4] However, Mishra S et al found SCC cells having the lowest mean cell perimeter^[6].

In current study, it was found that there was a gradual increase in nuclear area from normal to SCC with SCC having the highest mean nuclear area, which is consistent with other studies showing SCC having the highest mean nuclear area^[4,7,9] However, few studies revealed a different scenario, HSIL having the highest mean nuclear area^[6,8,10].

We also found observed a gradual increase in nuclear perimeter of cells, with SCC cells having the highest mean nuclear perimeter, comparable results were seen in some of the previous studies in literature^[7,9]. However, HSIL was seen having the highest mean nuclear perimeter in few other studies.^[6,8,10]

In the present study, it was found there was gradual increase in nuclear diameter from normal to reactive to SCC, except for ASC-H which had lower nuclear diameter from ASCUS and LSIL. Similar results were observed by Tiwari AK et al, with the exception of ASC-H, which had a smaller nuclear diameter than LSIL^[4], findings observed by Rani D et al also showed the mean nuclear diameter of SCC having the highest value.^[9]

However, few other studies^(5,10) conducted showed mean nuclear diameter of SCC less than HSIL. In a study done at kelambakkam, Tamil Nadu found that the mean nuclear diameter of LSIL was smaller than ASCUS and of SCC was less than HSIL^[10]. In a study done in

Wroclaw Medical University, Poland it was observed increase in mean nuclear diameter from normal to dysplastic cell, however they found HSIL having more nuclear diameter than SCC^[5].

Increase in nuclear to cytoplasmic ratio was observed in current study when normal pap smears compared to abnormal pap smears with SCC having the maximum N:C ratio. Our findings are in correlation with the study done by Tiwari AK et al^[4] and Mishra S et al⁽⁶⁾.

However in the study conducted by Mishra et al, ASC-H was having more N:C ratio than HSIL^[6].

Comparison between the individual groups was also done by unpaired t test and we found that on comparing reactive with ASCUS all the parameters were significant except for cell perimeter (p value = 0.211),

On comparing ASCUS with LSIL, we found N:C ratio to be statistically significant (p value 0.054) and other parameters were not statistically significant with cell area p value (0.076), cell perimeter (p value 0.372), nuclear area (p value 0.445), nuclear perimeter (p value 0.172) and nuclear diameter (p value 0.144).

On Comparing LSIL with ASC-H there was significant difference between cell area (p value 0.011), nuclear area (p value 0.021) and nuclear diameter (p value 0.008) but no significant difference between cell perimeter (p value 0.412), nuclear perimeter (p value 0.577) and N:C ratio (p value 0.100).

There was significant difference in nuclear area (p value 0.034) and nuclear diameter (p value 0.010) on comparing ASC-H with HSIL but no significant difference was found in cell area (p value 0.4), cell perimeter (p value 0.98), nuclear perimeter (p value 0.49) and N:C ratio (p value 0.071).

On comparing LSIL with HSIL we found cell perimeter (p value 0.427) and cell area (p value 0.014) to be more in LSIL than HSIL. The p value of the latter was found to be statistically significant. The

Bethesda system mentions that the cells of HSIL are smaller than LSIL. The nuclear area (p value 0.001), nuclear perimeter (p value 0.000), nuclear diameter (p value 0.000) and N:C ratio (p value 0.000) were more in HSIL than LSIL, the values of which were statistically significant. The Bethesda system states that degree of nuclear enlargement is more variable in HSIL than that seen in LSIL and N:C ratio is high in HSIL compared to LSIL^[11].

In our study we found nuclear area (p value 0.018), nuclear perimeter (0.006), nuclear diameter (0.017), N:C ratio (0.008) were more in SCC than HSIL, the values of which were statistically significant. However, cell area and cell perimeter were more in HSIL than SCC but it was not statistically significant (p value 0.812, 0.202). The Bethesda system also mentions that cells of SCC may be somewhat smaller than those of HSIL and nuclei of SCC demonstrates marked variation in nuclear area^[12].

Based on the statistical analysis, it can be said that all the groups differ from each other enough to permit the use of their morphometric characteristics for diagnostic purposes. The results of the tests show that there are many differences between the various groups of cells. This is confirmed by the statistical analysis as well. The cells belonging to each group of cytological changes can be identified on the basis of the morphometric characteristics measured, and this can be applied in diagnostic cytology.

Conclusion:

Diagnosis of cervical pre neoplastic and neoplastic lesions is subjective and requires a trained pathologist. Morphometry aims to make this process more objective with distinction being made on specifically selected parameters. So, this process can be automated and this technique can act as adjunct for use by clinical pathologist or for providing provisional diagnosis in remote areas with unavailability of trained pathologist.

However there are limitations of this study due to small sample size. Besides glandular cell abnormalities were not studied and reference ranges of morphometric parameters are also not available.

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