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Morphometry in the Cytological Diagnosis of Cervical Smears

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A – research concept and design; **B** – collection and/or assembly of data; **C** – data analysis and interpretation; **D** – writing the article; **E** – critical revision of the article; **F** – final approval of article; **G** – other

Abstract

Background. Morphometry of cells found in normal and abnormal smears taken from the vagina and the uterine cervix is the assessment of the size and diameter of their nuclei. The values of these quantities provide information on the origin of these cells and the degree of possible anomalies. Determining the morphometric traits of different types of cells found in the cervix and the uterus is a very important element in the diagnosis of disorders that often lead to cervical tumors.
Objectives. The aim of this research is to determine the morphometric characteristics of cells found in cervical smears by measuring the cell circumference, the diameter of the nucleus and the cell surface areain order to identify which clinical group the cells belong to, which facilitates diagnosis.
Material and Methods. The study material consisted of cervical smearsthat demonstrated the presence of cells in various phases of the clinical Bethesda classification. For each clinical classification, the values of the cell circumference, the cell surface area and thediameter of the nucleus were measured for 100 cells.
Results. The largest cells are normal cells in the surface layer. In relation to these cells, the atrophic cells fromthe groups containing atypical squamous cells of undetermined significance (ASC-US), low-grade squamous intraepithelial lesions (LSIL), high-grade squamous intraepithelial lesion (HSIL) and tumor cells tend to decrease in size, with small variations. Considering the mean values of the parameters analyzed, the cells of the LSIL group are larger than those from the ASC-US group. According to the mean values, normal cells have the smallest nucleusand the HSIL cells and tumor cells have the largest.
Conclusions. The statistical analysis shows significant differences between the morphometric traits in the different clinical groups, which indicates that morphometry can be used in cytological diagnosis (**Adv Clin Exp Med 2014, 23, 2, 289–293**).

Key words: morphometry, vaginal smears, cytodiagnosis, cervix uteri.

Morphometry of cells found in normal and abnormal smears taken from the vagina and the uterine cervix is an assessment of the size and diameter of their nuclei. The values of these quantities provide information on the origin of these cells and the degree of possible anomalies. Determining the morphometric traits of different types of cells found in the cervix and the uterus is a very important element in the diagnosis of disorders that often lead to cervical tumors. However, there are also other important factors in diagnosis, such as the cell system in the smear, the total number of cells, the number of nuclei, the number of nucleoli and chromatin placement in the nucleus. The presence of cells with specific characteristics indicates the clinical changes involved. The Bethesda System is the most commonly recommended classification system for cytological smears; it reflects the most current knowledge about irregularities in cervical smearsand is associated with new technologies for diagnostic testing that have emerged in the last decade [1–3].

Material and Methods

Material

The material consisted of cervical smears collected in the Department of Pathomorphology

and Oncological Cytology at the Wroclaw Medical Univerisity, Poland. Smears from different clinical groups were tested: normal smears, where the superficial cells and glandular cells were evaluated separately; smears showing the presence of atrophic cells; smears with atypical squamous cells of undetermined significance (ASC-US); smears with low-grade squamous intraepithelial lesions (LSIL) and high-grade squamous intraepithelial lesions (HSIL); and smears showing the presence of cervical tumors. In addition to these cell groups, atrophic cells (atrophic cells occur in smearsin which there is a lack of superficial cells) were also assessed. In some hormonal states, where there is a decrease in estrogen production in the body, cells of the intermediate and by-base layers are observed in the smear. The presence of greater or lesser numbers of polymorphonuclear neutrophil granulocytes is a characteristic feature of these hormonal states as well [4, 5]. The smears were tinted using the HE and the Papanicolaou methods. The study measured 100 cells for each of the stages in the Bethesda System.

Methods

The assessment of morphometric feature was done using the dotSlide system (Olympus, Poland). Using this imaging system, the cell circumferences, the cell surface area and diameter of the nuclei from each group were measured. The dotSlide system is not only a digital virtual microscope, but also an imaging system used in "virtual microscopy" – the digital equivalent of a conventional optical microscopy [6].

For normal cells, atrophic cells, groups of cells with ASC-US, LSIL and HSIL and tumor cells, the following statistical parameters were calculated for each of the 3 attributes (the cell surface area, cell circumference and the diameter of the nucleus): the average value, the minimum and maximum values, the median and standard deviation. These parameters are presented in Tables 1–6.

Statistical Analysis of Test Results

STATISTICA 9 software (StatSoft, Tulsa, USA) was used applied to perform the statistical analysis. In order to check whether there are statistically significant differences, it was necessary to check whether the distributions are normal or abnormal. The Shapiro-Wilk test was performed. Next, anonparametric Kruskal-Wallis ANOVA was carried

Table 1. Test results for normal smear (superficial cells)

Parameter	Cell circumference [μm]	Area [μm2]	Diameter of the nucleus [μm]
Average value	195.27	2382.16	8.48
Maximum value	270.84	4648.95	14.11
Minimum value	124.08	1027.16	4.55
Median	194.66	2324.37	8.73
Standard deviation	32.95	794.67	2.00

Table 2. Test results for atrophic cells

Parameter	Cell circumference [μm]	Area [μm2]	Diameter of the nucleus [μm]
Average value	105.68	768.40	8.98
Maximum value	192.54	2125.88	12.82
Minimum value	52.28	151.31	3.05
Median	101.46	643.58	9.11
Standard deviation	33.41	468.10	1.91

Table 3. Test results for ASC-US

Parameter	Cell circumference [μm]	Area [μm2]	Diameter of the nucleus [μm]
Average value	86.54	457.46	12.87
Maximum value	239.68	2596.59	22.92
Minimum value	47.59	150.35	7.09
Median	83.10	400.04	12.37
Standard deviation	25.75	284.38	2.87

Table 4. Test results for LSIL

Parameter	Cell circumference [μm]	Area [μm2]	Diameter of the nucleus [μm]
Average value	93.69	539.55	13.21
Maximum value	156.57	1292.86	20.77
Minimum value	52.78	181.10	7.03
Median	90.65	496.03	13.21
Standard deviation	22.81	245.56	2.71

Table 5. Test results for HSIL

Parameter	Cell circumference [μm]	Area [μm2]	Diameter of the nucleus [μm]
Average value	82.61	421.62	15.71
Maximum value	149.02	1299.28	26.51
Minimum value	49.03	163.39	9.84
Median	79.25	362.92	15.41
Standard deviation	21.59	207.78	2.90

Table 6. Test results for tumor cells

Parameter	Cell circumference [μm]	Area [μm2]	Diameter of the nucleus [μm]
Average value	61.30	254.50	13.30
Maximum value	88.36	531.60	22.83
Minimum value	46.79	147.86	8.42
Median	60.51	244.68	13.22
Standard deviation	8.47	67.87	2.44

out to determine the presence or absent of differences among the groups. The level of statistical significance was $\alpha = 0.05$.

Results

The Shapiro-Wilk test showed that for the cell surface area, each group was characterized by a normal distribution. For the cell circumference, the analysis showed that only the group of superficial cells had normal distribution. For the diameter of the nucleus, only the atrophic and LSIL groups had normal distribution. Based on the values calculated in the non-parametric Kruskal-Wallis ANOVA (the result for each attribute is $p < 0.001$ [Table 7]) and the box-and-whiskers plot (Fig. 1–3) it can be concluded that there are statistically significant differences between the groups. On the basis of the measurements and the statistical analysis, it can be seen that the largest measured cells are the superficial cells; the atrophic cells are smaller than the normal ones. The average, maximum, minimum and median values show that the diameter of the nuclei of cells derived from the ASC-US group is larger than the nuclei of normal cells. The wide range of cells and of cell nucleus sizes can confirm the presence of anomaliesin the ASC-US group. Large variations in the values of the area of the cells are also seen in the group of LSIL cells. HSIL cells are characterized by a small size compared to normal cells anda nucleus forming 3/4 of the cell area. Tumor cells are the smallest cells among the cell groups measured.

Discussion

Based on the statistical analysis, it can be concluded that all the groups differ from each other enough to permit the use of their morphometric characteristics for diagnostic purposes. Taking into account the cell area, superficial cells from the normal group had a much larger area than all the other groups, so they can easily be distinguished. Atrophic cells differ significantly from superficial cells, but are similar in area to cells from the ASC--US group. The other parameters – the cell circumference and the diameter of the nucleus – can also be used. The differences in the values of the cell circumference are not very large, but ASC-US cells have larger nuclei than atrophic cells. A similar situation can be seen in the case of atrophic cells compared to both LSIL and HSIL cells. The

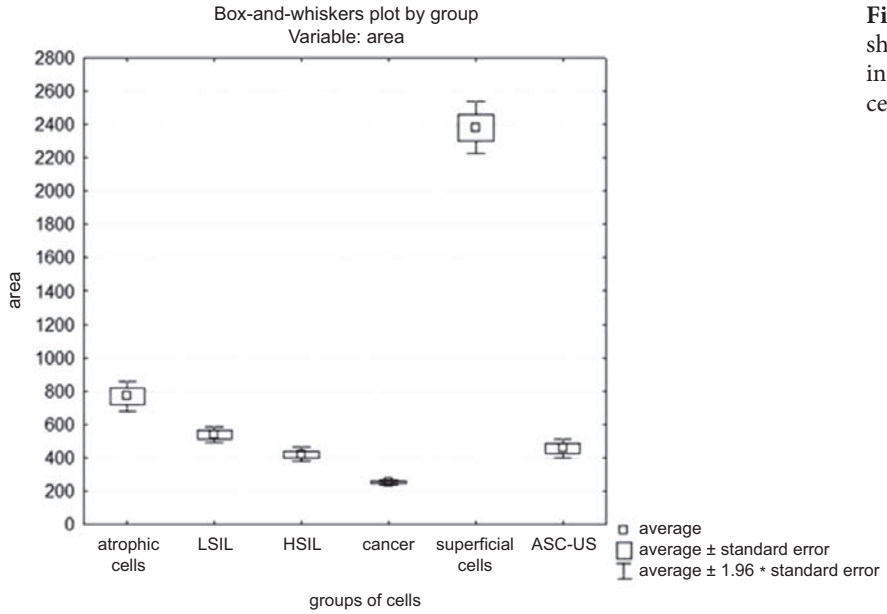

Fig. 1. Box-and-whiskers plot showing the arrangement of the individual groups of cells for the cell surface area parameter

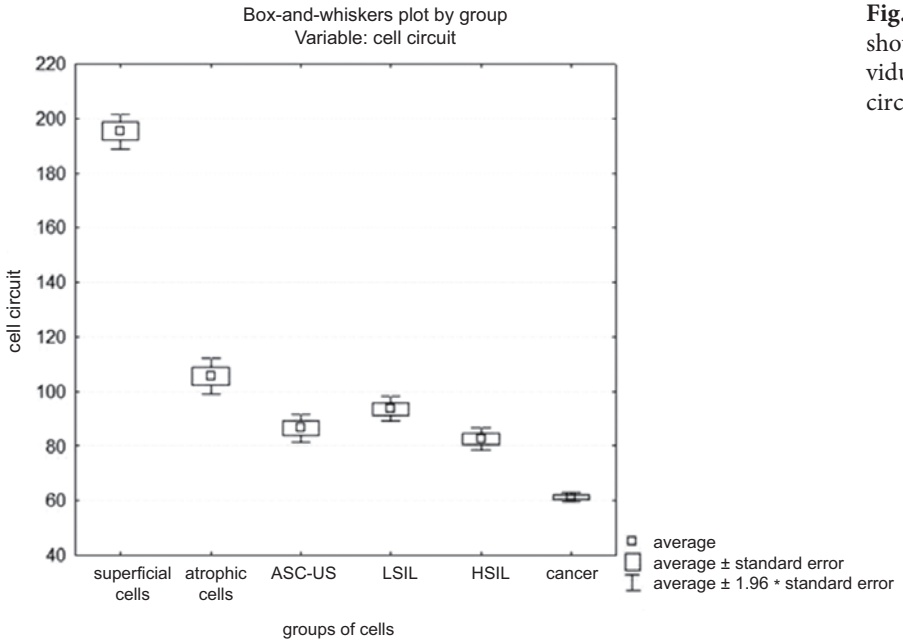

Fig. 2. Box-and-whiskers plot showing the arrangement of individual groups of cells for the cell circumference parameter

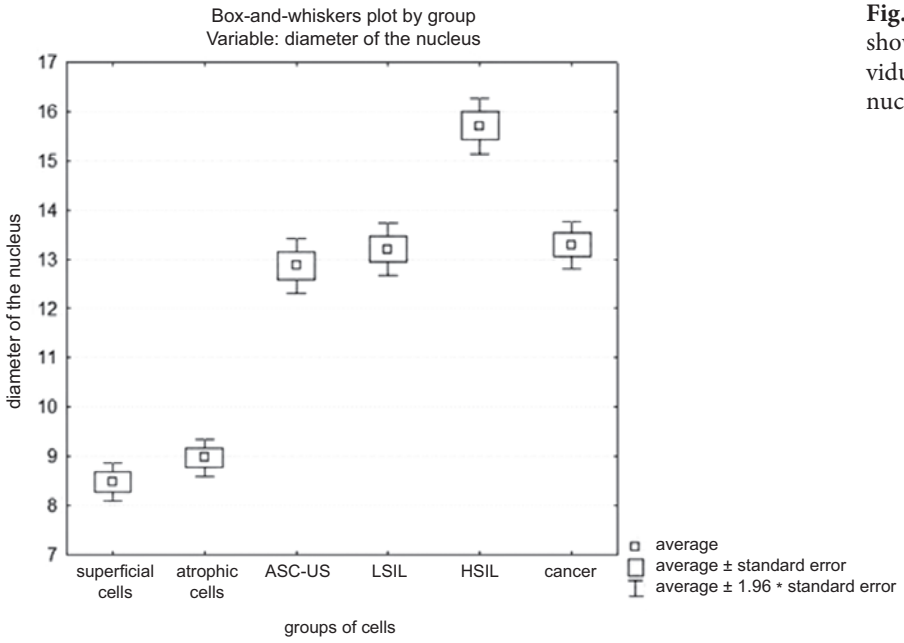

Fig. 3. Box-and-whiskers plot showing the arrangement of individual groups of cells for the cell nucleus diameter parameter

Table 7. Comparison of the cell groups by attribute: ANOVA rankKruskal-Wallis test results

Attribute	Value p level
Area	0.000
Cell circumference	0.000
Diameter of the nucleus	0.000

differences between these 2 groups can be seen only in the diameters of the nuclei. The last group to be compared with atrophic cells is the group of tumor cells. Based on the values obtained, each of the morphometric features can be used to differentiate these 2 groups. Comparingthe ASC-US and LSIL groups, it can be seen that the areas of these groups of cells are too similar to each other to allow their differentiation on this basis alone. Cells from these groups have similar nucleus diameters and cell circumferences, so the authors believe that their morphological characteristics need to be taken into consideration as well. To compare ASC-US cells and HSIL cells, the best parameter to differentiate them is the nucleus diameter, which is larger in the HSIL group, where they cover more than 3/4 of the cell area. Tumor cells can be distinguished from ASC-US cells using both the area and the cell circumference. As expected, the results for the LSIL and HSIL cells groups show that the values of the circumference and area of these groups are very similar; the difference can be seen in the diameter of the nucleus, which is significantly larger in the HSIL group. Tumor cells are much smaller in size than LSIL cells, and smaller than HSIL cells; in terms of area, tumor cells are half the size of HSIL cells. The diameter of the nucleus is smaller in tumor cells.

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. However, in some cases, these measurements do not give an unambiguous answer to the question which cytological changes are involved. The help of a skilled cytologist is still needed to dispel all doubts.

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