Original paper

Morphometric differentiation of squamous cell carcinoma and adenocarcinoma of the cervix

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The aim of the study was to define the morphometric characteristics of squamous cell carcinoma (SCC) and adenocarcinoma (AC) cells and identify opportunities to differentiate these tumors based on determination of the morphometric characteristics in order to use the results of research in the diagnosis of cervical cancer. Pap smears with two histopathologically confirmed cervix cancers were used for testing. For the morphometric assessment of cancer cells the dotSlide program was used. The mean nucleus area for AC cells was 126.62×10^{-6} m^2, while this value for SCC was 145.07×10^{-6} m^2 ($p = 0.000132$). The mean circumference of AC cells was 42.43×10^{-6} m, while for SCC cells it was 46.67×10^{-6} m ($p = 0.00$). AC cell diameter was 13.83 μm, whereas for SCC cells it was 15.36×10^{-6} m. On average the AC cell surface area was 195.72×10^{-6} m^2, while for SCC cells it was 249.94×10^{-6} m^2 ($p = 0.00565$). The mean AC cell circumference was 54.90 µm, whereas for SCC cells it was 65.23×10^{-6} m ($p = 0.00607$). The research shows that, despite the presence of statistically significant differences for each morphometric parameter, determination of cancer type cannot be used in the diagnosis.

Key words: morphometry, vaginal smears, squamous cell carcinoma, adenocarcinoma.

Introduction

Cervical cancer is one of the most common cancers found in women [1, 2] and accounts for about 12% of these tumors. Each year there are about 500 thousand new cases of cervical cancer among women. Most cases of cervical cancer occur in developing countries [3]. Particularly in those countries, cervical cancer is a huge health problem. One reason is the lack of a screening program [4]. In developed countries, in spite of the technological progress, 35% of patients are diagnosed with invasive cervical cancer [5]. The two most common types of cervical cancer are squamous cell carcinoma and adenocarcinoma. Squamous cell carcinoma constitutes 85% of all cases of cervical cancer [6, 7]. Squamous cell carcinoma can be classified according to its invasiveness or degree of differentiation. The incidence of adenocarcinoma has increased over the past four decades [8, 9, 10]. This cancer represents 15-35% of all cases of cervical cancer [11, 12]. The prognosis for adenocarcinoma is worse than for squamous cell carcinoma due to a worse response to radiotherapy and cancerous changes which are difficult to detect [13]. Most cases of cervical cancer are preceded by dysplastic changes with different degrees of progression. These changes involve an increase in the size of the cell nucleus and number of mitotic divisions and structural changes at the tissue and cell level [14, 15]. Dysplastic changes, not always, but change into cervical cancer [14, 16]. The cell nuclei of *in situ* cancer are pleomorphic. Chromatin is abundant and spread all over the surface of the nuclei. Invasive cancer cells are characterized by a significant increase in

pleomorphism. Nuclear membrane of cells has uneven edges. In the nucleus there occurs one or more prominent nucleoli. There are changes in the size and shape of the nucleus, as well as changes to its structure [1]. Morphometry of cells found in cervical smears allows one to assess the size and diameter of cells and their nuclei. The values obtained by measuring morphometric characteristics provide information about anomalies and origin of the cells. The aim of the study is to define the morphometric characteristics of the squamous cell carcinoma (SCC) and adenocarcinoma (AC) cells found in cervical smears. Measurements are used to identify opportunities of differentiation of these tumors based on determination of the morphometric characteristics. The aim is also determine whether the research results can be used in the diagnosis of cervical cancer.

Material and methods

The material consisted of cervical smears from the Institute of Pathomorphology Hist.-med. s.c. in Wrocław and was available thanks to the courtesy of Dr. Peter Prajs. Smears with histopathologically certified SCC and AC of the cervix were used for testing. 183 adenocarcinoma cells from four patients were assessed as well as 182 squamous cell carcinoma cells also derived from four patients. Smears were stained by hematoxylin-eosin (HE), Papanicolaou and Shorr methods. The local institutional review board granted approval of the study. Patient's informed consent was not necessary, because samples from the Institute of Pathomorphology Hist. med. s.c. were archival samples. For the morphometric assessment of cancer cells the dotSlide program (Olympus, Poland) was used. Using this program, the parameters of nuclei were measured: diameter, surface area, and circumference. Also analyzed were parameters such as circumference and surface area of the cell; however, due to the "sheets" of cancer cells in specimens, the number of measurements for these parameters was 24, both for squamous cell carcinoma and adenocarcinoma cells. Cells were chosen randomly from all over the slides. Cell were viewed and measured under magnification (40×). DotSlide is not only a digital virtual microscope but also an imaging system that later becomes the equivalent of a traditional optical microscope [17]. Statistical analysis was performed using STATISTICA, version 10 (StatSoft, Tulsa, USA). The significance level used in the tests was $\alpha = 0.05$. Two analyses were applied in testing. The first analysis was used for all the cells of tumors, while in the second analysis cells were divided into smaller groups, which were the individual patients. In the first analysis Student's t-test was used (t-test for independent trials), while in the second the Kruskal–Wallis non-parametric ANOVA was used.

Results

The two cancers were compared by evaluating each morphometric characteristic separately. The mean nucleus area for AC cells was 126.62×10^{-6} m^2, whereas this value for the SCC cells was 145.07×10^{-6} m^2 ($p = 0.000132$). The mean circumference of AC cells was 42.43×10^{-6} m, while for the SCC cells this value was 46.67×10^{-6} m ($p = 0.00$). AC cell diameter was 13.83×10^{-6} m, whereas for SCC cells this value was 15.36×10^{-6} m ($p = 0.00$). Two additional measurements were made for 24 cells. Measured characteristics were the area of the cell and cell circumference. The mean AC cell surface area was 195.72×10^{-6} m^2, while the SCC cell surface area was 249.94×10^{-6} m^2 (0.00565). The mean AC cell circumference was 54.90×10^{-6} m, while this value for AC cells was 65.23×10^{-6} m ($p = 0.00607$). In the second analysis, charts and categorized histograms were made. Charts and histograms showed differences between average values of parameters for groups of AC and SCC cancer cells. These differences are determined by the fact that cells come from different patients. Next, the ANOVA test was performed along with post-hoc comparisons indicating significant statistical differences ($p = 0.00$) between groups (patients). The probability of specifying the right type of cancer based on the cell derived from a patient was calculated on the basis of performed analysis (Table I).

Table I. Analysis 2 – The probability of specifying the right type of cancer based on the cell derived from a patient

	A max	B max	A min	B min	Z	p	p (%)
The surface of the nucleus	126.6151	145.0652	50.55937	40.03529	0.203656	0.581	58
The circuit of the nucleus	42.4258	46.6662	8.15419	6.57073	0.287972	0.613	61
The diameter of the nucleus	13.8258	15.3559	2.91715	2.61328	0.276677	0.609	61
The surface of the cell	195.7183	249.9404	72.95132	55.22293	0.423034	0.664	66
The circuit of the cell	54.8979	65.2342	12.24889	12.64076	0.415283	0.661	66

A – adenocarcinoma, B – squamous cell carcinoma, Z = (B max – A max)/(A min + B min), p – probability

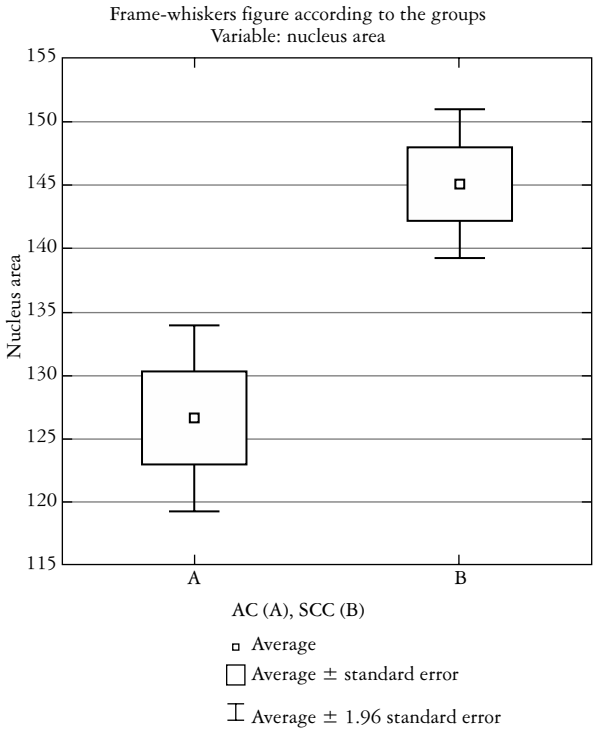

Fig. 1. Graph showing the deployment of individual groups of cells for the parameter of the surface area of the nucleus

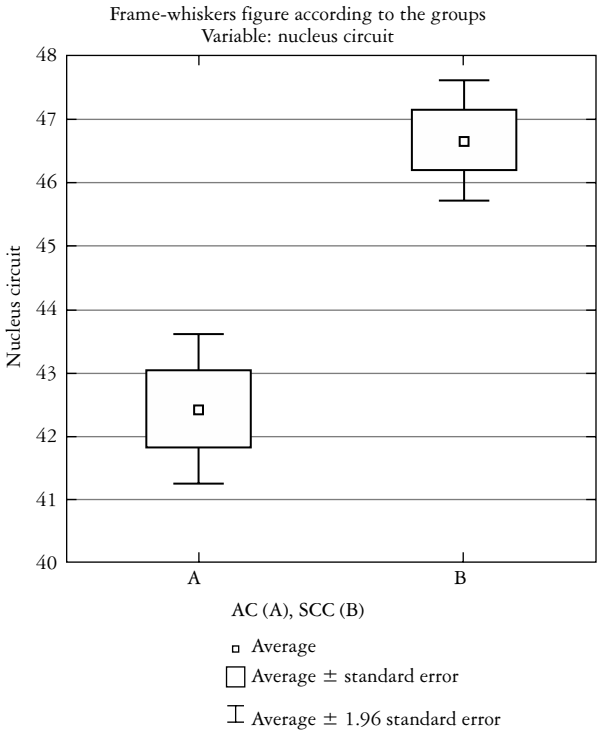

Fig. 2. Graph showing the deployment of individual groups of cells for the parameter of the circumference of the nucleus

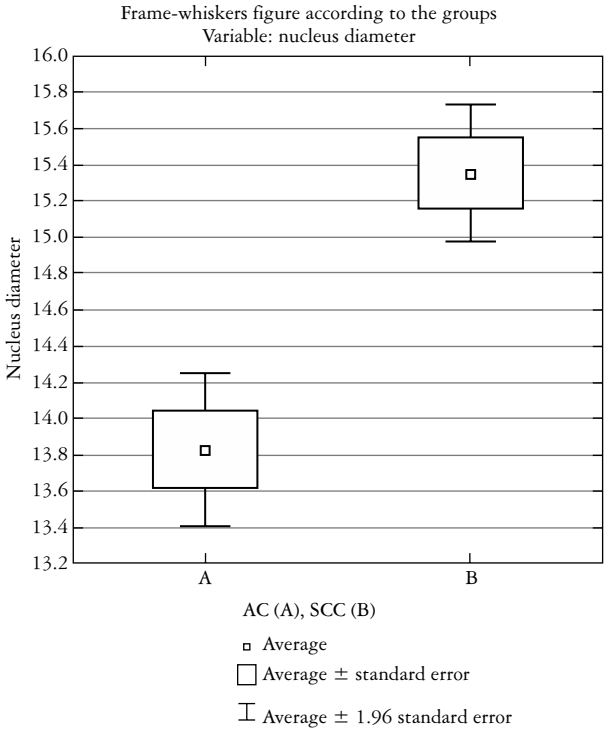

Fig. 3. Graph showing the deployment of individual groups of cells for the parameter of the diameter of the nucleus

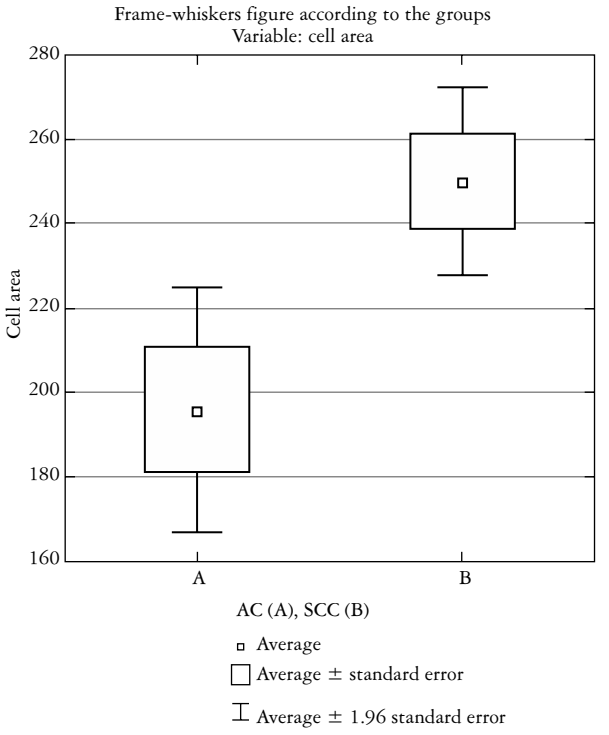

Fig. 4. Graph showing the deployment of individual groups of cells for the parameter of the surface area of the cell

Discussion

The p-value determined for each parameter in the first analysis was less than 0.05, i.e. the differences are statistically significant. On the basis of the Student t-test and graphs (Fig. 1-5) it can be assumed that differentiation of the two cancers is possible on the basis of each parameter. It is also possible to consider any combination of these parameters. We owe such solid results to the large sample size (without

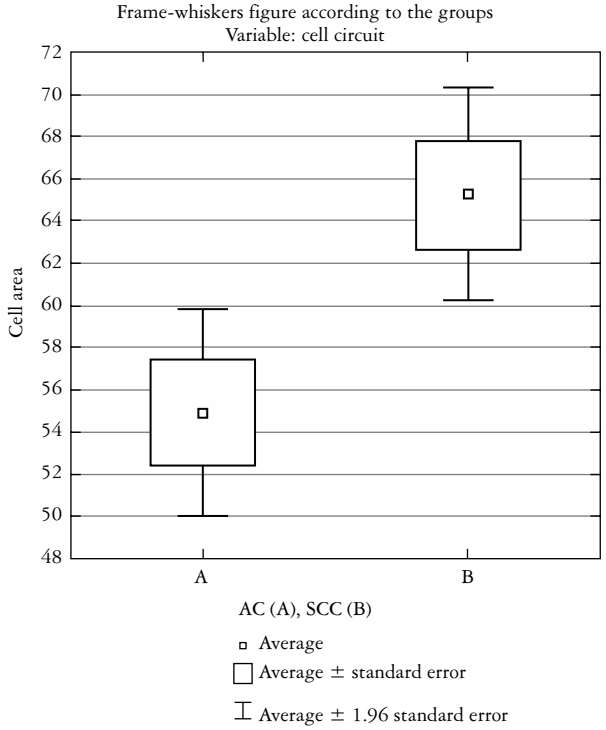

Fig. 5. Graph showing the deployment of individual groups of cells for the parameter of the circumference of the cell

taking into consideration two additional parameters, for which the number of cells is 24). These results cannot be a reason to deduce a specific type of cancer on the basis of a single cell measurement. The graphs show the scatter of the results for both cancers. Adenocarcinoma cells have a greater scatter for all of the parameters. The second analysis aimed to show the allowable possibility of making a mistake when assigning a particular type of cancer to a specific cell. As mentioned earlier, the differences between average values of parameters for groups of AC and SCC cancer cells arise from the fact that the cells come from different patients. The performed analysis shows that the probability of specifying the right type of cancer on the basis of a single parameter is within the limits from 58% to 66%, depending on the parameter.

The research shows that, despite the existence of statistically significant differences for each morphometric parameter, determining whether we are dealing with adenocarcinoma or squamous cell cancer is uncertain. The probability of correct determination of the type of cancer is around 60%, and it is not a strong basis for a correct diagnosis. This supports rejecting morphometry as a potential diagnostic method in differentiation of cervical cancer and its application in diagnostics of this tumor.

The authors declare no conflict of interest.

References

1. Gonzalez-Oliver A, Echeverria OM, Hernandez-Pando R, et al. Ultrastructural study of the nuclei of normal, dysplastic, and carcinomatous epithelial cells of the human cervix uteri. Ultrastruct Pathol 1997; 21: 379-392.
2. Parkin DM, Bray F, Ferlay J, et al. Global cancer statistics 2002. CA Cancer J Clin 2005; 55: 74-108.
3. Ferlay J, Bray F, Pisani P. Globocon 2000: cancer incidence, mortality and prevalence worldwide. Version 1.0 IARC Cancer Base No. 5. IARC Press, Lyon 2001.
4. Landoni F, Maneo A, Colombo A, et al. Randomized study of radical surgery versus radiotherapy for stage Ib-IIa cervical cancer. Lancet 1997; 350: 535-540.
5. Disaia PJ, Creasman WT. Cervical Cancer in Clinical Gynecologic Oncology. Disaia PJ, Creasman WT (eds.). Mosby Yearbook, St. Louis 1997; 1-106.
6. Altekrufs SF, Lacey JV, Brinton LA. Comparsion of human papilloma virus genotypes, sexual and reproductive risk factors of cervical adenocarcinoma and squamous cell carcinoma. Northeastern United States. Obstet Gynecol 2003; 188: 657-663.
7. Rutledge FN, Galakatos AE, Wharton JT, et al. Adenocarcinoma of the uterine cervix. Am J Obstet Gynecol 1975; 122: 236-245.
8. Epstein E, Di Legge A, Masback A, et al. Sonographic characteristics of the squamous cell cancer and adenocarcinoma of the uterine cervix. Ultrasound Obstet Gynecol 2010; 36: 512-516.
9. Bray F, Carstensen B, Moller H, et al. Incidence trends of adenocarcinoma of the cervix in 13 European countries. Cancer Epidemiol Biomarkers Prev 2005; 14: 2191-2199.
10. Vizcaino AP., Moreno V, Bosch FX, et al. International trends in the incidence of cervical cancer: I. Adenocarcinoma and adenosquamous cell carcinomas. Int J Cancer 1998; 75: 536-545.
11. McLellan R, Dillon MB, Woodruff JD, et al. Long-term follow-up of stage I cervical adenocarcinoma treated by radical surgery. Gynecol Oncol 1994; 52: 253-259.
12. Miller BE, Flax SD, Arheart K, et al. The presentation of adenocarcinoma of the uterine cervix. Cancer 1993; 72: 1281-1285.
13. Chung J, Kim M, Cho NH, et al. T2-weighted fast spin-echo MR findings of adenocarcinoma of the uterine cervix: comparison with squamous cell carcinoma. Yonsei Med J 1999; 40: 226-231.
14. Robbins S. Patologia humana. Iberoamericana, Mexico 1990.
15. Fu SY, Reagan WJ, Ralph MR. Definition of precursors. Gynecol Oncol 1981; 12: 220-234.
16. Scully ER. Definition of precursors in gynecologic cancer. Cancer 1981; 48: 531-537.
17. Olympus [Internet]. Polska [cited 2015 Apr 5]. Available from: http://www.olympus.pl/.

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