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Evaluation of the Quantitative Cytological Changes in the Epithelium of the Uterine Cervix

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Abstract: *The cytological investigation is a subjective method which does not allow to determine accurately the morphological changes of the epithelium cells, therefore additional diagnostic methods must be used. Such a method is the Quantitative DNA Analysis. We used the obtained results for statistical processing in order to determine whether there is a significant difference between the cell populations with different degrees of dysplasia and in cases of carcinoma of the uterine cervix. No such differences were registered between the diploid cells in phases of DNA presynthesis and synthesis. Significant difference was calculated when we compared aneuploid cells. This means that cell populations with different degrees of dysplasia can be distinguished by the aneuploid cells in the smear.*

Keywords: *dysplasia/cancer, quantitative DNA analysis, diploid/aneuploid cells, histogram, statistics.*

1. Introduction

Cancer of the uterine cervix is the most common malignant neoplasm in women in the developing countries. It ranks second in the world with 500,000 new cases diagnosed annually [1].

The cytological gynecological investigation is the basic method for observing the early and reversible states of the disease. Its purpose is to register the cytological changes in the epithelium. The method is based on the observation of the morphological cell characteristics such as shape, size, nuclear-cytoplasmic ratio, as well as the shape, size, location and the number of nucleoli, if such are present. Another important characteristics for the accurate classification of the cellular changes are the structure and distribution of the nuclear chromatin and DNA content [3].

The morphological changes evaluated by a cytologist which makes their classification subjective. In order to eliminate the subjectiveness in the cytological practice new methods had to be developed. One of them is the Quantitative DNA analysis which helps registration of abnormal cells, thus eliminating the possibility for misdiagnosis. The ploidy status of the cell population can be determined and evaluated with the current methods of Image Analysis [3].

This article presents the results of the quantitative investigation in different cases of precancerous lesions and cancer of the uterine cervix.

2. Materials and Methods

The obtained cytological material is prepared as a smear.

2.1. Quantitative DNA Analysis (DNA cytometry)

Determining the change in the amount of the nuclear DNA is of primary significance for the accurate classification of the registered cellular changes. For this purpose an automatic system for DNA analysis – CAS ™ 200D Beckton-Dickenson, IL, USA was used. The cytological material is stained by the Feugen method in such a way that the stain is absorbed by the chromatin, while the cell's cytoplasm and nucleoli, remain unstained (Fig.1). This means that the density and intensity of the nuclear staining corresponds to the DNA content [4].

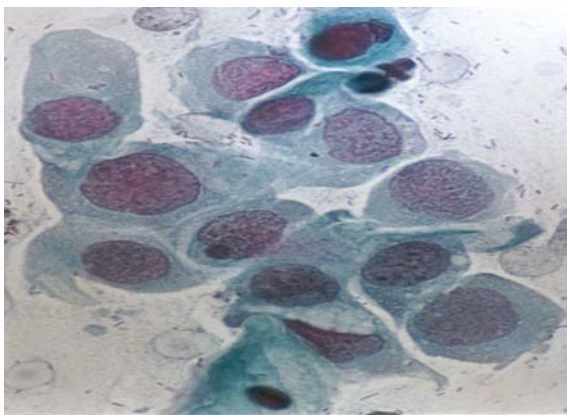
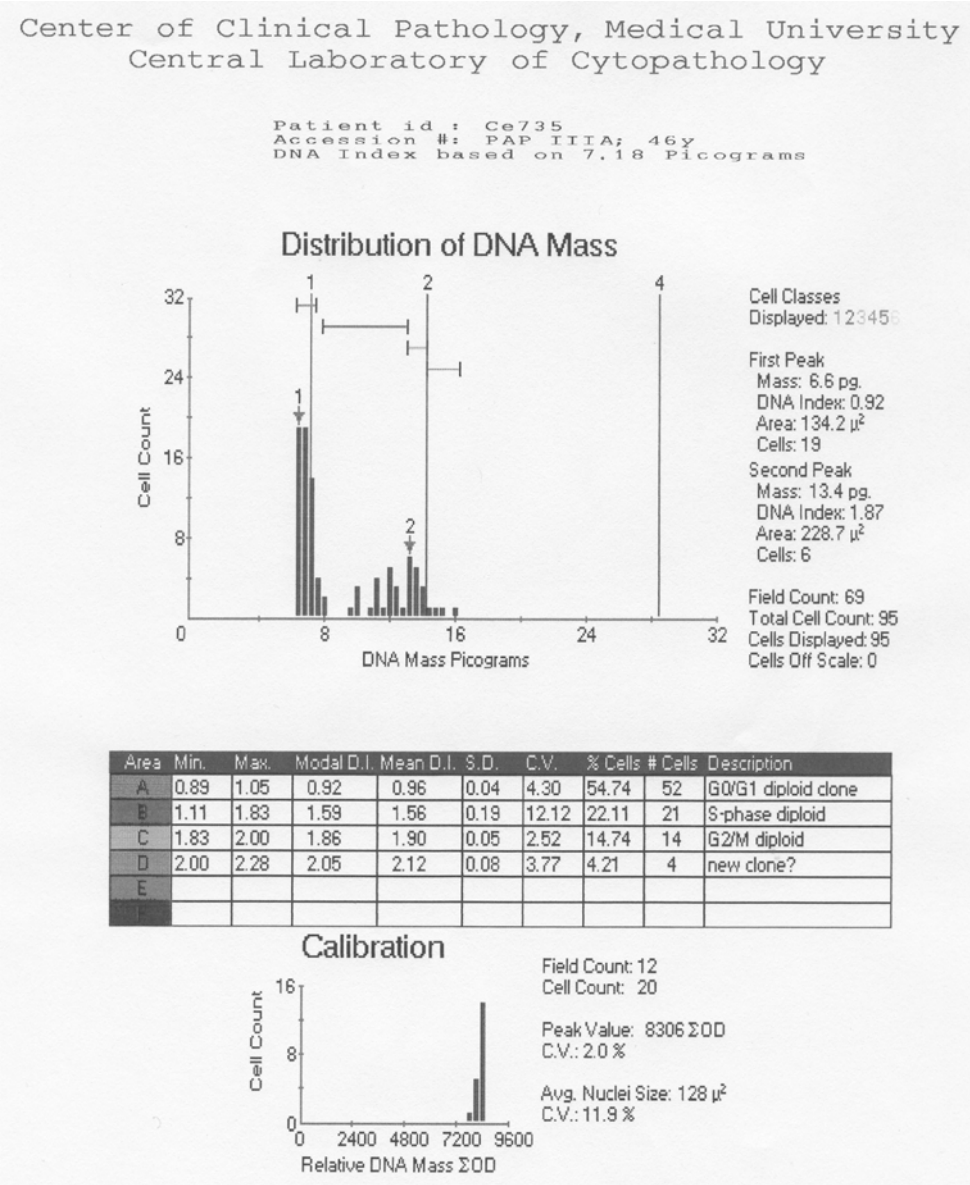


Fig. 1. Cells in case of severe dysplasia

About 100-200 tumor cells are measured in a standard quantitative investigation, which is used to determine the distribution of the DNA in the particular tumor population. The system's software allows classification and separation of the examined nuclei according to their parameters by using different morphometric filters and gives the opportunity to choose those nuclei that can be used for the analysis. The obtained results are presented as a histogram (Fig.2) showing the final DNA distribution in the investigated material [2].

When the cell population is homogenous, i.e. it consists of cells of the same type its histogram is unimodal. This means that cells containing equal amounts of DNA are grouped in the histogram. The non-homogenous popu-lations (Fig. 2) respectively, consist of cells in different phases of the cell cycle, therefore their histograms consist of



Area	Min.	Max.	Modal D.I.	Mean D.I.	S.D.	C.V.	% Cells	# Cells	Description
A	0.89	1.05	0.92	0.96	0.04	4.30	54.74	52	G0/G1 diploid clone
B	1.11	1.83	1.59	1.56	0.19	12.12	22.11	21	S-phase diploid
C	1.83	2.00	1.86	1.90	0.05	2.52	14.74	14	G2/M diploid
D	2.00	2.28	2.05	2.12	0.08	3.77	4.21	4	new clone?
E									
F									

Fig. 2. DNA distribution in case of mild dysplasia. Cells are separated within the cycle, based on their DNA content

two or more peaks. In this case the distribution is bimodal or polymodal [2].

Determining the total DNA content allows tracing deviations and facilitates classification of the lesions as inflammatory or malignant [6].

2.2. Statistical analysis

To find out the deviations between different stages the obtained results were statistically processed.

The issue in the quantitative cytological investigations is whether and how much do cell groups differ from each other based on the measured parameters. The evaluation of the difference between two groups is based on the differences between some parameters, characterizing them. Such a parameter is the mathematical expectation μ of the cell population [2].

Let n_1 be the number of cells measured in group 1, and n_2 be the number of cells measured in group 2. In case that the average and the standard deviation of the cell populations is unknown a priori, the difference in their mathematical expectations is evaluated by the formula:

$$t = \frac{\bar{x}_{n_1} - \bar{x}_{n_2}}{\sqrt{\frac{n_1 s_1^{\,2} + n_2 s_2^{\,2}}{n_1 + n_2 - 2}\left(\frac{1}{n_1} + \frac{1}{n_2}\right)}}, \quad (1)$$

where $\bar{x}_{n_1}$ and $\bar{x}_{n_2}$ are the average values of the measured parameter, and $s_1^{\,2}$ and $s_2^{\,2}$ are the statistical deviations in the two groups.

It is well known from the mathematical statistics that t has a distribution of Student with $f = n_1 + n_2 - 2$ degrees of freedom. To determine the significance of the difference, a level of significance α is chosen and the value of $t\alpha$ with $P(t \geq t\alpha) = \alpha$ is found from the t-distribution table at the corresponding f. If t is greater than $t\alpha$, the difference $\mu_1 - \mu_2$ is significant, thus we conclude that the hypothesis that the two samples come from different populations is true and the reliability of this inference is $P = 1 - \alpha$ [2].

3. Results and Discussion

18 samples were used for the practical investigation of the changes in the DNA quantity in cases of lesions with different degrees of dysplasia and cancer of the uterine cervix. Because of the different amount of cytological material in the samples different number of nuclei ranging from 27 to 205 were measured. The results obtained are presented in Tables 1-4.

Normally a histogram consists of non-dividing cells or G_0 – cells, cells in phase of synthesis (S-phase), meaning that they are undergoing replication, and cells in which this process is completed and the DNA content is doubled – G_2 cells [3]. Because of disturbances in the genome in cell populations with dysplastic and tumor changes we registered aneuploid DNA values. This leads to the appearance of new and well distinguished peaks in the histogram and it shifts towards greater quantity values for the nucleic acid [5, 7, 8].

It seems that as the changes in the genome intensify, i.e. when the degree of dysplasia increases one can see that in the histogram cells in different phases group around the same place. This means that cells in different phases of the cell cycle contain equal amounts of DNA. This on the other hand makes statistical processing difficult.

Tabl. 1 shows that in PAP IIIA (cases of mild dysplasia) all samples but one show no significant difference between each other. This allows these cells to be combined in

one general population so they can be compared to other cells in the same phase but with a different degree of dysplasia.

Table 1. Significance evaluation in case of mild dysplasia

Sample number	878	945	537	735	686
878	–	+	++	–	++
945	+	–	–	–	–
537	++	–	–	–	–

Here "–" is insignificant difference and "+" stands for significant difference. The significance at $\sigma = 0.05$ and 0.025 is denoted by "+" and "++", respectively.

The results for group IIIB are shown in Tabl. 2 where all but one cases with moderate dysplasia show no significant difference.

Table 2. Significance evaluation in case of moderate dysplasia

Sample number	1616	1617	1413	980
1616	–	–	–	++
1617	–	–	–	–
1413	–	–	–	–

Tabl. 3 shows the results from group IV. Here two out of three samples of severe dysplasia show significant difference, therefore they cannot be combined in a common group.

Table 3. Significance evaluation in case of severe dysplasia

Sample number	2990	1242	2932
2990	–	0.95	–
1242	0.95	–	++
2932	–	++	–

In group V there was a significant difference between all three carcinoma samples (Tabl. 4).

Table 4. Significance evaluation in case of carcinoma of the uterine cervix

Sample number	2477	2949	2699
2477	–	++	++
2949	++	–	++
2699	++	++	–

If the difference between the compared groups is insignificant they can be combined and the calculation of their new average values and the average standard deviations can be done according to equations (2) and (3):

$$\bar{z} = \frac{n_1\bar{x} + n_2\bar{y}}{n_1 + n_2}, \tag{2}$$

$$\sigma_z^{\,2} = \frac{1}{n_1 + n_2 - 1}[(n_1 - 1)\sigma_x^{\,2} + (n_2 - 1)\sigma_y^{\,2} + \frac{n_1 n_2}{n_1 + n_2}(\bar{y} - \bar{x})^2]. \tag{3}$$

The results from the between group comparison of data show that there is no significant difference between diploid cells with mild and moderate dysplasia, compared by their presynthetic (G_1) and synthetic phases.

However a significant difference between cells in postsynthetic phase has been registered. There wasn't such a difference between the new cell clones. No significant difference was observed when comparing diploid cells with mild dysplasia in G_1 phase with cells with severe dysplasia.

Therefore diploid cell populations with different degrees of dysplasia cannot be distinguished by their presynthetic phase or phase of synthesis, but could be distinguished by their postsynthetic phase. When we compared the aneuploid populations the results showed that only in G_0/G_1, there was not a significant difference. Such difference we registered in S-phase (0.95) which increased to 0.99 in the next phase.

4. Conclusion

The quantitative DNA analysis should be used as an objective method for the cytological investigation. Significant differences between the cell populations in different cases of dysplasia are registered when comparing aneuploid cells. This means that the precancerous lesions can be distinguished from one another by their aneuploid populations where genome changes intensify. We were not able to find a quantitative pattern for each degree of dysplasia probably because of the small number of smears that we have used.

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Оценка на количествените цитологични промени в епитела на шийката на матката

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(Р е з ю м е)

Цитологичните изследвания са субективен метод, който не позволява точното определяне на морфологичните промени на епителните клетки. Затова е необходимо използването на допълнителни методи, които да елиминират погрешното диагностициране. Такъв е методът на количествения DNA анализ. Получените резултати са използвани за статистическа обработка при определяне на това, дали съществува значителна разлика между популациите на клетки с различна степен на дисплазия и в случаи на карцином на шийката на матката. Не са регистрирани такива разлики между диплоидни клетки във фазата на пресинтез и синтез. Била е отчетена значителна разлика, когато са сравенени анеиплодни клетки. Това означава, че клетъчни популации с различна степен на дисплазия могат да се открият по техните анеиплоидни клетки.