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Cytomorphometric Analysis of Cervical Papanicolaou Smear for Females with Gynecological Clinical Complaints

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Abstract

Background: Limited information is provided on the quantitative cytomorphometric study of the cervical Pap test. The cervical Pap test is an important screening program for cervical cancer. A quantitative cytomorphometric examination of cervical Pap is used to accurately identify precancerous and cancerous lesions early and to reduce the occurrence and avoidance of invasive cancer. This study was aimed to assess the cytomorphological parameters (nuclear diameter [ND], cytoplasm diameter [CD], and nuclear-to-cytoplasmic ratio [N/C ratio]) of squamous epithelial cells from a cervical Pap smear.

Methods and Results: A prospective study was performed on 142 consecutive cervical Pap smears from women with gynecological clinical complaints. The ND and CD were determined by the Optika optical microscope camera using a digitizer cursor in both axes. The final images were taken with an X40 magnification. The ND, CD, and the N/C ratio were then measured and expressed in micrometers.

The women were classified into 5 age groups: 5(3.5%) in the age group of <19 years, 46(32%) in the 20-29 group, 67(47.2%) in the 30-39 group, 23(16.2%) in the 40-49 group, and 1(0.7%) woman was over age 50. There were no significant differences in the N/C ratio of superficial cells between age groups. The ND, CD, and the N/C ratio were significantly higher in women with clinical complaints than in women without clinical complaints

Conclusion: Cytomorphometic analysis might assist in the identification of cellular alterations due to gynecological diseases and increase the sensitivity and accuracy of the Pap smear technique.(**International Journal of Biomedicine. 2021;11(1):46-49.**)

Key Words: cytomophometry • Pap smear • nuclear diameter • cytoplasm diameter • nuclear-to-cytoplasm ratio

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Introduction

A Pap test is an important screening procedure for cervical cancer. The identification of precancerous lesions by the Pap test decreases the occurrence of precancerous lesions and avoids the development of invasive cancer.(1) The background of the Pap test, developed by George Papanicolaou, the "father of cytology," has shown that specific cervix cells have morphological characteristics that can be used to diagnose carcinoma. Cells are obtained from the cervix, the lower part of the uterus protruding into the vagina.(2) The most mature squamous cell is considered a superficial cell, typically polygonal (45-50 μm in diameter), with a pyknotic nucleus (5-6 μm in diameter). Intermediate cells are also mature squamous

cells, typically polygonal (20-40 µm in diameter), with vesicular nucleus dimensions of 6-9 µm in diameter, and sometimes binucleated. The immature cells are named parabasal and basal cells, the parabasal cells are circular or oval rather than polygonal (15-30 µm in diameter), the nucleus is variable in size and typically larger than that of the intermediate cell. Parabasal and basal cells are not normally examined because the Pap test does not scrape off the entire thickness of the epithelium, just a few upper layers.[3] Exfoliative cytology is focused on epithelial tissue physiology. Usually, the epithelium is routinely exfoliated, and cell surface and thickness loss are permanent.[4] Ordinary epithelial cells are coherent in nature, but they shed or exfoliate as they mature. During infection and malignancies, exfoliation is excessive and epithelial cell morphology varied.[5] Cytomorphometry is a quantitative approach focused on the calculation of cytomorphological parameters, including the nuclear diameter (ND), cytoplasm diameter (CD), and the nuclear-to-cytoplasmic ratio (N/C ratio), which may improve the efficiency of the cytology.[6] Most of the published research addresses cytomorphological analysis for buccal smear[7,8] or morphometric study for histological samples of the cervix;[9] few studies discuss cytomorphological investigation for the Pap smear. The current research was therefore intended to evaluate the quantitative analysis for the cytomorphological parameters of the exfoliative, epithelial squamous cell in the cervical Pap smear.

Materials and Methods

A prospective study was performed on 142 consecutive cervical Pap smears from women with gynecological clinical complaints. The scraped materials were taken with a cyto-spatula, smeared onto a glass slide, and immediately fixed in 95% ethyl alcohol. Subsequently, the smears were stained by the Papanicolaou staining technique.

Papanicolaou staining method

Slides were dehydrated by a descending concentration of alcohol (95%, 70%) and distilled water for 3 minutes each. They were treated with Mayer's Hematoxylin for 5 minutes for nuclear stain and blued for 10 minutes in flowing tap water. The smears were counterstained with orange G6 for 5 minutes, differentiated in 95% ethanol for 10 seconds, then treated with Eosin EA50 for cytoplasmic stain for 5 minutes and again differentiated in 95% ethanol for 10 seconds. Finally, the smears were dehydrated through ascending concentration of alcohol (70%, 95%, 100%) for 3 minutes each, cleared in xylene, and mounted in Distrene Polystryne Xylene. The cells were examined by using X40 objective lenses.[10]

Cytomorphometric analysis

Ten clearly defined cells with good staining were chosen by systematic sampling stepwise by shifting the microscope stage in a Z shape from left to right in order to avoid measuring the same cell again. Any folded, clumped, or distorted nucleus or cell was avoided in each smear. The superficial, intermediate and parabasal cells were established by morphology and staining characteristics. The ND and CD were determined by the Optika optical microscope camera using a digitizer cursor in both axes. The final images were taken with an X40 magnification. The ND, CD and N/C ratios were then measured and expressed in micrometers. Quality management measures were adopted during the collection and processing of samples.

Statistical analysis was performed using the statistical software package SPSS version 20.0 (IBM Corp., Armonk, N.Y., USA). Continuous variables were presented as mean±standard deviation (SD). Means of 2 continuous normally distributed variables were compared by independent samples Student's t-test. Multiple comparisons were performed with one-way ANOVA. The frequencies of categorical variables were compared using Pearson's chi-squared test. A value of $P \leq 0.05$ was considered significant.

The ethics and research committee approved the study, and consent forms were obtained from all patients prior to procedures.

Results

A total of 142 smears from women aged from 16 to 49 years were examined. The women were classified into 5 age groups: 5(3.5%) in the age group of <19 years, 46(32%) in the 20-29 group, 67(47.2%) in the 30-39 group, 23(16.2%) in the 40-49 group, and 1(0.7%) woman was over age 50. Unsatisfactory smears (n=14) were excluded from the study. The quantitative cytomorphological analysis is shown in Table 1, Figure 1, and Figure 2.

Table 1.

Cytomorphometric parameters (µm) in superficial (s) and intermediate (i) cells

Type of cells	n	Minimum	Maximum	Mean±SD
NDs	128	2.2	9.00	4.9±1.7
CDs	128	17.2	62.00	37.5±10.6
N/Cs	128	0.05	0.24	0.1±0.03
NDs	117	3.0	18.60	6.9±2.6
CDi	117	13.6	57.40	32.2±10.1
N/Ci	117	0.1	0.57	0.23±0.07
NDi	5	6.0	10.00	8.0±1.6
CDi	5	23.0	35.00	29.0±5.3
N/Ci	5	0.9	0.43	0.9±0.1

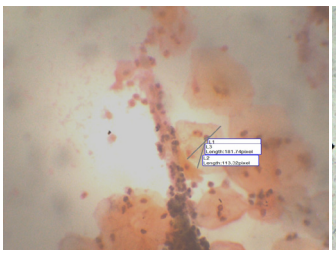

Fig. 1. *Cervical Pap smear. Measuring the CD and N/C ratio using OPTIKA software.*

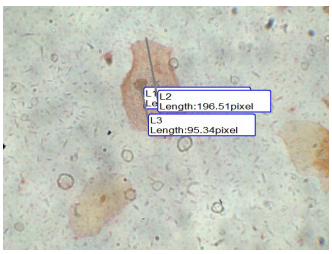

Fig. 2. *Cervical Pap smear. Measuring the ND and N/C ratio using OPTIKA software.*

There were no significant differences in the N/C ratio of superficial and intermediate cells between age groups

(Table 2). The ND, CD, and the N/C ratio were significantly higher in women with clinical complaints than in women without clinical complaints (Table 3). There were significant differences in ND, CD, and N/C ratio according to type of clinical complaints (Table 4).

Table 2.

The N/C ratio for superficial and intermediate cells according to the age groups

Type of cells	Age group (years)	n	Mean±SD	Minimum	Maximum	*P*-value
Superficial	< 19	4	0.1±0.02	0.09	0.14	0.39
	20-29	44	0.1±0.03	0.05	0.24	
	30-39	60	0.1±0.03	0.07	0.23	
	40-49	20	0.1±0.03	0.07	0.17	
Intermediate	< 19	4	0.2±0.09	0.13	0.25	0.89
	20-29	38	0.2±0.08	0.12	0.44	
	30-39	58	0.2±0.8	0.14	0.57	
	40-49	17	0.2±0.06	0.14	0.38	

Table 3.

Cytomorphometric parameters (CMP) according to routine Pap for women with or without clinical complaints

Type of cells	CMP	Clinical complaints	Mean±SD	*P*-value
Superficial cells	ND	Yes	7.0±1.1	0.001
		No	4.7±1.6	
	CD	Yes	41.6±6.6	0.081
		No	37.1±10.8	
	N/C ratio	Yes	0.17±0.03	0.003
		No	0.1±0.03	
Intermediate cells	ND	Yes	9.4±3.2	0.002
		No	6.7±2.1	
	CD	Yes	35.7±5.6	0.306
		No	31.96±10.27	
	N/C ratio	Yes	0.275±0.126	0.047
		No	0.221±0.069	

Table 4.

Cytomorphometric parameters for superficial cells according to type of clinical complaints

Superficial cell	n=128	ND	CD	N/C
Lower abdominal pain with vaginal discharge	57	4.5±1.5	36.3±11.7	0.1±0.03
Infertility	38	5.4±1.6	38.4±9.9	0.5±0.02
Fistula	3	5.6±0.3	44.1±7.9	0.1±0.02
Uterine fibroid	3	5.7±2.3	38.7±14.6	0.2±0.03
Irregular cycle	12	4.2±1.4	40.4±11.0	0.1±0.02
Routine Pap	9	7.2±0.9	41.9±6.9	0.2±0.03
Oophoritis	3	4.5±2.1	31.2±8.3	0.1±0.1
Syphilis	2	2.8± 0.0	24.9±1.6	0.1±0.02
Polycystic ovaries	1	3.20	25.8	0.124
P-value		0.001	0.28	0.001

Discussion

Cytomorphometric testing or image processing of exfoliated cells has also been proposed as a main measure to the classification and identification of cell and nuclear variations in these cytological smears, indicating that this computer-assisted analysis of microscope images can improve the susceptibility of exfoliative cytology to early detection of oral cancer, as these techniques are reliable, accurate and precise. The Pap test is the most effective cancer screening test in the world, although it has a high incidence of false-negative results due to subjective interpretation.

The current research attempted to calculate the ND, CD, and N/C ratios by computer-assisted, morphometric analysis in cervical exfoliative cytology using Optika software. In this study, there were no substantial differences in the N/C ratio of superficial cells between age groups, and this is inconsistent with previous studies conducted by Anuradha and Sivapathasundharam.[11] Patel et al.[12] observed that basal cells could only divide by a set of numbers; therefore, tissue renewal ability decreases with age, leading to aged cell accumulation. Cells that remain for a longer period of time contribute to the effects of various local environmental factors, which can be clarified by both estrogen and progesterone increase at puberty, accompanied by an increase and decrease in the uterine cycle stage. The level of these hormones declines at post-menopause. These hormones are responsible for the rise in ND, CD, and N/C ratio.[11,12]

In our research, the ND and N/C ratio were statistically significant among women at routine Pap clinics and women with clinical complaints. This finding explains that most gynecological complaints relate to fertility hormones and later promote the growth, differentiation, and maturation of the squamous epithelium of the cervix.[13]

A number of diseases, inflammation, and reactive conditions have contributed to a change in cell morphology. Many causes, such as cervicitis, have been accompanied by loss of cervical columnar cells, a typical aspect of the maturation process. As a result, sexually transmitted infections are hypothesized to encourage maturation through inflammation activation and subsequent cellular repair. In comparison, oral contraception has been associated with increased cervical ectopy (or less cervical maturation). Some have indicated that the presence of increased immaturity is due to hormonal contraception, which induces tissue edema and endocervical eversion.[13] Rani et al.[14] reported that cytomorphometry parameters were statistically significant between precancerous and cancerous Pap smears. Cigarette smoking is another potential influence because nicotine and its metabolites can be found in cervical mucus.[15] Interestingly, both hormonal contraceptives and longtime smoking have been correlated with an induced risk of cervical cancer,[16,17] and HPV infection is widespread in adolescents and young adults.[18]

In conclusion, it was evident that cytomorphometic analysis might assist in the identification of cellular alterations due to gynecological diseases and increase the sensitivity and accuracy of the Pap smear technique.

Limitations of the study

Only 142 females participated in this research, so for further studies, the sample size should be expanded in order to improve the precision and accuracy of the findings by examining each of the gynecological problems and making associations with alterations in cervical squamous cells, while also taking into account age and other environmental factors.

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Competing Interests

The authors declare that they have no competing interests.

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