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Interobserver Variability in Cervical Smears from Patients with a History of Abnormal Cytology: Comparison of Conventional Pap Smears and Liquid-Based Cytology

Anormal Sitoloji Hikayesi Olan Hastalarda Servikal Sitoloji Değerlendirmesinde Gözlemciler Arası Değişkenlik: Geleneksel Pap Smear ve Sıvı Bazlı Sitoloji Karşılaştırması

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ORIGINAL INVESTIGATION
ÖZGÜN ARAŞTIRMA

ABSTRACT
ÖZET

Objective: One of the problems encountered when assessing cervical precancerous lesions is intra- and interobserver variability. The aim of this study was to determine the degree of interobserver variation in conventional PAP smears (CPS) and liquid-based cytology (LBC).

Materials and Methods: The diagnostic variability among three pathologists was assessed using 120 smears (67 conventional CPS and 53 LBC). The cases were selected retrospectively from the archives of the Pathology Department among the patients with follow-up, such as biopsy confirmation and/or persistent/resolving disease in the follow-up smear. The observers examined the slides in a blinded fashion.

Results: Diagnostic agreement on the presence or absence of intraepithelial lesions was found in 30 of 51 slides (58.82%) of LBC (kappa=0.42) and in 44 of 67 slides (65.67%) of conventional cytology (kappa=0.50). The agreement was slightly higher in conventional smears. The highest agreement was in the LSIL category with a kappa value of 0.50 in LBC and 0.62 in conventional cytology, while ASCUS was the least reproducible diagnosis.

Conclusion: Our results are in agreement with the literature in that the reproducibility of cervical cytology shows low to moderate consistency. The study showed no significant difference between LBC and CPS in the reproducibility of the diagnosis.

Key words: Cytological technique, intraepithelial neoplasia, cervical, cervical smear

Amaç: Servikal prekanseröz lezyonlar sözkonusu olduğunda tanının tekrarlanabilirliği problemlerden biri olarak karşımıza çıkmaktadır. Çalışmamızda geleneksel pap smear ve sıvı bazlı sitoloji de gözlemciler arasındaki farlılık derecesinin belirlenmesi amaçlanmıştır.

Gereç ve Yöntemler: Yüz yirmi smear (67 konvansiyonel, 53 sıvı bazlı sitoloji) incelenerek, 3 patolog arasındaki tanısal değişkenlik araştırılmıştır. Olgular patoloji departmanı arşivinden, biyopsi ile kanıtlı, gerilemiş veya ısrarcı hastalık şeklinde takipli hastalar arasından seçilmiştir. İnceleme kör olarak yapılmıştır.

Bulgular: İntraepitelyal lezyon varlığı veya yokluğu konusundaki tanısal uzlaşma, sıvı bazlı sitolojilerde %58,82 (kappa=0,42) geleneksel yaymalarda %65,57 (kappa=0,50) olarak bulundu. Sonuçlar geleneksel yaymalarda biraz daha yüksek olup en yüksek tutarlılık LSIL kategorisinde (geleneksel yaymada kappa 0,62, sıvı bazlı sitolojide 0,50) izlenirken ASCUS uyumun en düşük olduğu tanı olarak belirlendi.

Sonuç: Sonuçlarımız literatür bilgilerine paralel olup, servikal sitolojide tekrarlanabilirlik düşük-orta derecededir. Bu çalışmada sitoloji yöntemleri arasında belirgin fark gözlenmemiştir.

Anahtar kelimeler: Sitolojik teknik, intraepitelyal neoplazi, servikal, servikal smear

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Introduction

Cervical cytology is one of the best cancer screening programs, resulting in a dramatic decrease in the incidence of cervical cancer in many developed countries since conventional Pap smears (CPS) began to be used for cervical cancer screening in the 1960s (1). However, it must be kept in mind that many factors influence the success of this screening program, and the disparities in diagnostic assessment of cervical cytology and the inaccuracy of cytological diagnosis have emerged as being very important (2).

Liquid-based cytology (LBC) is an alternative technique for transferring the cellular material collected from the transformation zone of the uterine cervix. In contrast to CPS, the cells are not directly spread on a slide, but rather into a vial containing fixative liquid (3). The commonly used automated LBC techniques are ThinPrep™ (Cytyc Corporation, Boxborough, MA, USA), SurePath™ (TriPath Imaging, Burlington, NC, USA), PapSpin™ (ThermoElectron, Pittsburgh, PA, USA), DNACITOLIQ (Digene Brazil, Sao Paulo, Brazil), and Liqui-Prep™ (LGMInternational, Fort Lauderdale, FL, USA) (4, 5).

The reproducibility of cervical cytological interpretations are low to moderate, and there are many studies with conflicting results regarding the superiority of LBC to CPS in this context (1, 3-8).

This paper focuses on the reproducibility of diagnostic classification between the use of CPS and LBC among three pathologists.

Materials and Methods

Case Selection and Evaluation

In this retrospective study, cervical cytology specimens were re-evaluated in 120 smears (67 CPS and 53 LBC) from 90 patients. Biopsy confirmation was present in 61 of them. The cases were selected retrospectively from the archives of the Pathology Department among the patients with follow-up, as biopsy confirmation and/or persistent/resolving disease in the follow-up smear.

The observers were three pathologists (two of with 7 years of experience as pathology specialists (first and second pathologists) and one professor of pathology (third pathologist) with 15 years of experience and a gynecopathology subspeciality) and the slides were examined blindly. Two slides were not examined by the second pathologist, so the total number of LBC in the statistical evaluation was 51 in this group.

PapSpin™ Procedure

The specimens were collected using a cervix brush. The brush was first smeared on a glass side for a CPS, then the head of the brush was removed and placed in the PapSpin™ preservation fluid and submitted to the laboratory. Some of the patients had only CPS, while a group had only LBS.

The vial containing the head of the brush was vigorously shaken using a vortex for 5-10 seconds. For specimens containing blood or mucus, 0.2-2.0 mL of cleaning solution were added. Then the contents of each vial were transferred to a megafunnel, centrifuged and transferred onto glass slides in a 21x14 mm rectangle. The slide was then fixed in alcohol for 10 minutes and Pap stained.

Data and Statistics

All pathologists independently examined the slides, blinded to the diagnosis made on the CPS or the previous reported diagnosis. The cytological interpretation was classified into eight categories: negative for epithelial abnormality (NEA); atypical squamous epithelial cells of undetermined significance (ASCUS); ASCUS having a few cells suspicious of low grade (LSIL) squamous intraepithelial lesion (ASCUS-L); atypical squamous epithelial cells with a high grade squamous intraepithelial lesion (HSIL) that cannot be excluded (ASC-H); LSIL; LSIL with a few cells suspicious of HSIL (LSIL-H); HSIL; and suboptimal smear (SUBOP).

Interobserver variability was tested using weighted kappa statistics and Fleiss' kappa statistics. Specifically, the weights were 0-0.19: very low accordance, 0.20-0.39: low accordance, 0.40-0.59: moderate accordance, 0.60-0.79: good accordance, 0.80-1.00: excellent accordance. The percentage of cases with diagnostic agreement between pathologists was reported.

Results

Among the 90 selected cases, 37 had CPS, 23 had LBC, and 30 had both LBC and CPS preparations.

The findings for LBC and CPS are summarized in Table 1 and Table 2, respectively. There was a slightly better agreement in the CPS preparations. The triple agreement percentage was 58.82% (30/51) in LBC with a kappa value of 0.427 and 65.67% (44/67) in CPS with a kappa value of 0.505. The highest agreement between diagnosis was in the LSIL group (kappa=0.625), while the lowest one was in the ASCUS group (kappa=0.045) (Table 3 and Table 4).

Table 1. The distribution of 51 cases diagnosed as negative or positive for intraepithelial abnormalities and epithelial abnormalities in liquid-based cytology preparations

Third pathologist			Second pathologist		Total
			Negative	Positive	
Negative	First pathologist	Negative	10	2	12
		Positive	7	4	11
		Total	17	6	23
Positive	First pathologist	Negative	1	0	1
		Positive	7	20	27
		Total	8	20	28

Table 2. The distribution of 67 cases diagnosed as negative or positive for intraepithelial abnormalities and epithelial abnormalities in conventional Pap smear preparations

Third pathologist			Second pathologist		Total
			Negative	Positive	
Negative	First pathologist	Negative	13	3	16
		Positive	7	6	13
		Total	20	9	29
Positive	First pathologist	Negative	1	2	3
		Positive	4	31	35
		Total	5	33	38

Table 3. The results of liquid-based cytology preparations according to three pathologists

	Second pathologist								
First pathologist	**NEA**	**ASCUS**	**ASC-H**	**ASCUS-L**	**LSIL**	**LSIL-H**	**HSIL**	**SUBOP**	**Third pathologist**
NEA	9	0	0	0	0	0	0	1	NEA
ASCUS	3	2	0	0	1	0	0	2	NEA
ASC-H	1	0	0	0	0	0	0	0	NEA
ASCUS-L	0	0	0	0	2	0	0	1	NEA
LSIL	0	0	0	1	0	0	0	0	NEA
LSIL-H	0	0	0	0	0	0	0	0	NEA
HSIL	0	0	0	0	0	0	0	0	NEA
SUBOP	1	0	0	0	0	0	0	0	NEA
NEA	0	0	0	0	0	0	0	0	ASCUS
ASCUS	2	1	0	0	0	0	0	0	ASCUS
ASC-H	0	0	0	0	0	0	0	1	ASCUS
ASCUS-L	0	0	0	0	0	0	0	0	ASCUS
LSIL	0	0	0	0	1	1	0	0	ASCUS
LSIL-H	0	0	0	0	0	0	0	0	ASCUS
HSIL	0	1	0	0	0	0	0	0	ASCUS
SUBOP	0	0	0	0	0	0	0	0	ASCUS
NEA	0	0	0	0	0	0	0	0	ASCH
ASCH	0	1	0	0	0	0	0	0	ASCH
ASC-H	0	0	0	0	0	0	0	0	ASCH
ASCUS-L	0	0	0	0	0	0	0	0	ASCH
LSIL	0	1	0	1	0	0	0	0	ASCH
LSIL-H	0	0	0	0	0	0	0	0	ASCH
HSIL	0	0	0	0	0	1	0	0	ASCH
SUBOP	0	0	0	0	0	0	0	0	ASCH
NEA	1	0	0	0	0	0	0	0	LSIL
ASCUS	2	0	0	0	0	0	0	1	LSIL
ASC-H	0	0	0	0	0	0	0	0	LSIL
ASCUS-L	0	0	0	0	0	0	0	0	LSIL
LSIL	0	0	0	0	8	1	0	0	LSIL
LSIL-H	0	0	0	0	0	0	0	0	LSIL
HSIL	0	0	0	0	0	0	0	0	LSIL
SUBOP									
NEA	0	0	0	0	0	0	0	0	LSILH
ASCUS	1	0	0	0	0	0	0	0	LSILH
ASC-H	0	0	0	0	0	0	0	0	LSILH
ASCUS-L	0	0	0	0	0	0	0	0	LSILH
LSIL	0	0	0	0	0	0	0	0	LSILH
LSIL-H	0	0	0	0	0	0	0	0	LSILH
HSIL	0	0	0	0	0	0	0	0	LSILH
SUBOP	0	0	0	0	0	0	0	0	LSILH
NEA	0	0	0	0	0	0	0	0	HSIL
ASCUS	1	0	0	0	0	0	0	0	HSIL
ASC-H	0	0	0	0	0	0	0	0	HSIL
ASCUS-L	0	0	0	0	0	0	0	0	HSIL
LSIL	0	0	0	0	0	1	0	0	HSIL
LSIL-H	0	0	0	0	0	0	0	0	HSIL
HSIL	0	0	0	0	0	0	0	0	HSIL
SUBOP	0	0	0	0	0	0	0	0	HSIL

NEA: Negative for intraepithelial abnormalities. ASCUS: Atypical squamous cells of undetermined significance. ASCUS-L: ASCUS having few cells suspicious of a low grade squamous intraepithelial lesion. ASC-H: Atypical squamous cells, HGSIL cannot be excluded. HSIL: High grade squamous intraepithelial lesion. LSIL: Low grade squamous intraepithelial lesion. SUBOP: suboptimal smear

The kappa values of interobserver variability varied between 0.366-0.567 with a moderate degree of agreement. The results are presented in Table 5.

Discussion

In order to evaluate the interobserver reproducibility of cervical smears prepared by conventional Pap smear or by the PapSpin™ method, a set of 120 cervical smears from 90 patients were evaluated by three pathologists. The interobserver variability showed a moderate degree of agreement with a slightly higher percentage in CPS. There have been several studies investigating interobserver variability in the diagnosis of cervical epithelial cell abnormalities including a large group by comparing CPS and LBC. In their study of a group of 20,000 patients, Yobs et al. (9) found 82-96.8% agree-

Table 4. The results of conventional Pap smear preparations according to three pathologists

	Second pathologist								
First pathologist	**NEA**	**ASCUS**	**ASC-H**	**ASCUS-L**	**LSIL**	**LSIL-H**	**HSIL**	**SUBOP**	**Third pathologist**
NEA	8	0	1	0	0	0	0	1	NEA
ASCUS	3	0	1	2	0	0	0	0	NEA
ASC-H	1	0	1	0	0	0	0	1	NEA
ASCUS-L	11	0	0	0	0	0	0	1	NEA
LSIL	0	0	0	0	0	0	0	0	NEA
LSIL-H	0	0	0	0	0	0	0	0	NEA
HSIL	0	0	2	0	0	0	0	0	NEA
SUBOP	1	0	0	1	0	0	0	4	NEA
NEA	0	0	0	2	0	0	0	0	ASCUS
ASCUS	1	1	0	0	0	0	0	1	ASCUS
ASC-H	0	0	1	1	0	0	0	0	ASCUS
ASCUS-L	0	0	0	0	1	0	0	0	ASCUS
LSIL	0	0	0	1	1	0	0	0	ASCUS
LSIL-H	0	0	0	0	0	0	0	0	ASCUS
HSIL	0	0	0	0	1	0	1	1	ASCUS
SUBOP	0	0	0	0	0	0	0	0	ASCUS
NEA	0	0	0	0	0	0	0	0	ASCH
ASCH	0	0	0	0	0	0	0	1	ASCH
ASC-H	0	0	0	0	0	0	0	0	ASCH
ASCUS-L	0	0	0	0	0	0	0	0	ASCH
LSIL	0	0	0	0	0	0	0	0	ASCH
LSIL-H	0	0	0	0	0	0	0	0	ASCH
HSIL	0	1	0	0	0	0	0	0	ASCH
SUBOP	0	0	0	0	0	0	0	0	ASCH
NEA	1	0	0	0	0	0	0	0	LSIL
ASCUS	0	0	0	0	0	0	0	0	LSIL
ASC-H	0	0	0	0	0	0	0	0	LSIL
ASCUS-L	0	2	0	0	0	0	0	0	LSIL
LSIL	0	0	1	0	8	2	0	0	LSIL
LSIL-H	0	0	0	0	0	0	0	0	LSIL
HSIL	0	0	0	0	0	1	0	0	LSIL
SUBOP	0	0	0	0	0	0	0	0	LSIL
NEA	0	0	0	0	0	0	0	0	LSILH
ASCUS	0	0	0	0	0	0	0	0	LSILH
ASC-H	0	0	0	0	0	0	0	0	LSILH
ASCUS-L	0	0	0	0	0	0	0	0	LSILH
LSIL	0	0	0	0	0	0	0	0	LSILH
LSIL-H	0	0	0	0	0	0	0	0	LSILH
HSIL	0	0	0	0	0	0	0	0	LSILH
SUBOP	0	0	0	0	0	0	0	0	LSILH
NEA	0	0	0	0	0	0	0	0	HSIL
ASCUS	1	0	0	0	0	0	0	0	HSIL
ASC-H	0	1	0	0	0	0	0	0	HSIL
ASCUS-L	0	0	0	0	0	0	0	0	HSIL
LSIL	0	0	0	0	0	0	0	0	HSIL
LSIL-H	0	0	0	0	0	0	0	0	HSIL
HSIL	0	0	0	0	1	2	3	0	HSIL
SUBOP	0	0	0	0	0	0	0	0	HSIL

NEA: Negative for intraepithelial abnormalities. ASCUS: Atypical squamous cells of undetermined significance, ASC-H: Atypical squamous cells, HGSIL cannot be excluded, HSIL: High grade squamous intraepithelial lesion, LSIL: Low grade squamous intraepithelial lesion, ASCUS-L: Atypical squamous cells of undetermined significance, LSIL cannot be excluded, LSIL-H: Low grade squamous intraepithelial lesion, HGSIL cannot be excluded, SUBOP: Suboptimal smear

ment, with the lowest percentage in moderate dysplasia. Duca et al. (2) studied 120 CPS slides assessed by three cytotechnicians and found interobserver agreement with kappa values in the range of 0.418–0.575. Similarly, Klinkhamer et al. (10) noted 83.3% consistency with no more than one grade of disagreement. Confortini et al. (11), in their study reviewing a set of 100 slides assessed by 16 cytopathologists, found a moderate to good degree of agreement with kappa values varying from 0.35-0.57; the best agreement was seen in severe dysplasia. The results were similar in the case of CPS reproducibility. However, when compared with LBC, there are conflicting results. While some studies state a high degree of reproducibility with LBC, there are also studies showing no difference or less reproducibility (4-8, 12, 13).

Table 5. Percentage of agreement and weighted kappa scores between pathologists

	Method	Agreement	Kappa
First pathologist - Second pathologist	Liquid-based cytology	68.6%	0.366
Second pathologist - Third pathologist	Liquid-based cytology	72.5%	0.450
First pathologist - Third pathologist	Liquid-based cytology	73.5%	0.456
First pathologist - Second pathologist	Conventional Pap smear	76.0%	0.463
Second pathologist - Third pathologist	Conventional Pap smear	79.0%	0.567
First pathologist - Third pathologist	Conventional Pap smear	80.5%	0.493

In our study, we found a slight better agreement in the CPS preparations. The triple agreement percentage was 58.82% (30/51) in LBC with a kappa value of 0.427 and 65.67% (44/67) in CPS with a kappa value of 0.505. The highest agreement between diagnosis was in the LSIL group (kappa=0.625), while the lowest value was in the ASCUS group (kappa=0.045). These differences may be due to the LBC method. In a study using PapSpin™ as the LBC method (4), the results showed no great differences. However, the consensus of opinion is that the highest agreement is achieved in HSIL carcinoma, while the lowest agreement is in the ASCUS group (2, 5-9, 11, 12). Our results agree with this consensus opinion.

Conclusion

The interobserver reproducibility of cervical cytology is moderately independent from the method used. LBC is more comfortable for the pathologist but has a higher cost. The selection of technique will be made according to the socio-economic status of the patient and the country. However, screening of the population should be as broad as possible, regardless of which method is used.

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Conflict of interest

No conflicts of interest were declared by the authors.

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