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Review article

COMPARISON OF CONVENTIONAL PAP SMEAR AND LIQUID-BASED CYTOLOGY IN DETECTING CERVICAL ABNORMALITIES

Šečerović Đelila
University of Sarajevo, Faculty of Health Studies, Laboratory Technologies, Sarajevo, Bosnia and Herzegovina

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Abstract

Cervical cancer represents one of the most common types of cancer in women, requiring early diagnosis to reduce prevalence and mortality rates. The Pap smear plays a crucial role in the early diagnosis of precancerous lesions. While the conventional Pap smear has been the standard method for lesion detection, liquid-based cytology (LBC) is emerging as an alternative with potential advantages.

Research comparing the conventional Pap smear to LBC has shown that LBC yields a higher percentage of satisfactory samples and demonstrates greater sensitivity and specificity in identifying various cervical abnormalities. Similar findings have been reported in Japanese research. However, some studies have shown conflicting results, emphasizing the specificity of the conventional method. While some studies suggest that the conventional Pap smear is better at detecting ASC-US, others show similar or favorable results for the LBC method.

The LBC method stands out for its higher diagnostic sensitivity, especially in detecting various types of cervical abnormalities, while the conventional Pap smear method maintains its specificity, particularly in diagnosing LSIL. The LBC method has the advantage of better sample representativeness and fewer unsatisfactory samples.

Keywords: cervical cancer, Pap smear, liquid-based cytology, precancerous lesions of cervical cancer, diagnosis of cervical cancer, Pap smear classification.

INTRODUCTION

Cervical cancer (lat. *Carcinoma cervicis*) is a malignant condition that develops slowly over several years, allowing for detection in its early stages. Compared to other types of cancer, the diagnosis of cervical cancer is relatively straightforward and effective. Timely diagnosis, through regular Pap smear tests, enables the detection of early stages of cervical cancer (1).

The Pap smear is the most effective method for the early diagnosis of cervical cancer. According to the study by Derya et al. (2), participants who underwent Pap tests were more aware of gynecological cancers than those who did not (2).

The Pap smear test and liquid-based cytology (LBC) are the methods most commonly used for the early detection of precancerous lesions of the cervix (Latin: neoplasma malignum cervicis uteri). Conventional Pap smear has been the gold standard for cervical cancer screening for many years, but limitations in its sensitivity and specificity have led to the development and increased use of LBC (3). The association of human papillomavirus (HPV) infection with the development of cervical cancer has been established and is considered one of the most common causes. Two types of HPV, 16 and 18, are responsible for the majority of high-grade cervical precancerous conditions (4).

According to data from 2020, cervical cancer is the fourth most commonly diagnosed cancer and the fourth leading cause of cancer death in women, with an estimated 604.000 new cases and 342.000 deaths worldwide. About 90% of new cases and deaths worldwide in 2020 occurred in low- and middle-income countries. Globally, more than 500.000 new cases of cancer and 250.000 fatal cases are registered annually (5, 6).

Pap smear – Papanicolaou method or exfoliative cytopathology is the study of normal and altered states of spontaneously exfoliated or mechanically displaced cells with the aim of detecting and diagnosing various infections, abnormal hormonal activities, and precancerous or cancerous lesions (7).

The classification of Pap smear has evolved gradually throughout history, with advancements in technology and understanding of the biological nature of cervical dysplasia and cancer.

Previously, Pap smears were classified according to a system based on classes from I to V, according to the Papanicolaou classification (4). Its classification was based on the qualitative assessment of cell atypia. Although this classification was simple, it had the potential for different interpretations, which was its drawback. Nevertheless, the classification played a significant role in the history of cytology, as it was extremely important for saving a large number of lives. After numerous clinical studies worldwide, in 1954, the National Cancer Institute (NCI) recommended that the Papanicolaou classification should no longer be used for establishing a final diagnosis but continue to be used as a routine classification for triaging detected changes (5, 8).

The classification based on cervical intraepithelial neoplasia (CIN) is used to assess precancerous lesions in cervical cells. It is divided on a scale from 1 to 3 (9).

- CIN I (dysplasia gradus laevis) - indicates a mild precancerous lesion with a small number of altered cells of the squamous epithelium of the cervix. The cells have abundant, clear, well-defined cytoplasm. Based on type, the cells belong to superficial and intermediate squamous cells. The nucleus is enlarged compared to intermediate cells, and the chromatin is finely granular and moderately hyperchromatic (10).
- CIN II (dysplasia gradus medii) - moderate dysplasia with a variable number of altered cells. Large superficial, fewer intermediate, and small parabasal cells are present. They are mostly oval or round but can also be spindle-shaped. They exhibit surface maturation of cytoplasm, which stains cyanophilic, but there can also be many cells with eosinophilic cytoplasm. Nuclei are enlarged, and chromatin is moderately hyperchromatic. The N:C ratio is increased (11).
- CIN III (dysplasia gravis or carcinoma in situ) - severe dysplasia with a large number of abnormal cells or carcinoma in situ. Atypical parabasal cells occupy more than two-thirds of the total epithelium. They have scant cytoplasm forming a ring around the nucleus. Cells are round or oval, irregular, or elongated in shape. The nucleus is enlarged and hyperchromatic, with coarsely granular chromatin. The N:C ratio is extremely increased, making it easily recognizable (11).

The CIN classification is supplemented by dividing into low-grade squamous intraepithelial lesions (LSIL) and high-grade squamous intraepithelial lesion (HSIL) (4). Squamous intraepithelial lesion (SIL) is usually a result of sexually transmitted HPV, although SIL itself cannot be

transmitted from person to person. SIL is also termed dysplasia or neoplasia. It is divided into high-grade and low-grade SIL. CIN is graded as I, II, and III (3, 12).

LSIL indicates the presence of mild abnormalities in squamous cells, indicating a precancerous condition. These abnormalities usually regress spontaneously. However, in some cases, these changes can progress and lead to cancer development. Changes are typically caused by infection with certain types of HPV and are detectable on Pap smear. Most detected HPV infections are low-grade and regress spontaneously within two years. However, some LSIL cases progress to HSIL within two years, which is more likely in older women. LSIL is also called mild dysplasia, encompassing CIN I (4, 13, 14).

Routine classification for triage of detected changes in LSIL:

a) Changes associated with HPV,
b) Mild dysplasia CIN I (4).

HSIL represents the presence of abnormalities in squamous cells associated with HPV. It includes terms CIN II, CIN III, moderate and severe dysplasia, and carcinoma in situ. HSILs are associated with persistent infection and a higher risk of progression to invasive cancer, especially if the persistent infection is a high-risk genotype such as HPV16 and/or HPV 18 (15).

Routine classification for triage of detected changes in HSIL:

a) Moderate dysplasia CIN II,
b) Severe dysplasia CIN III,
c) Carcinoma in situ CIN III (4).

Cervical cell sampling for the Pap smear is performed by scraping the endocervical and exocervical areas. The primary aim is to sample the entire transformation zone (TZ) with minimal trauma to the cervical and endocervical epithelium. This is crucial as most precancerous changes occur within the TZ, making cell collection from this area extremely significant. Various devices are available for collecting cells from the cervix, such as Ayer's spatula, Szalay, plastic spatulas of different sizes, and cotton swabs (16).

In the early 1980s, research was initiated aimed at improving cytological preparations, leading to the development of liquid-based cell collection before placing them on slides. The result was liquid-based preparations. Finally, in the 2000s, the liquid-based cytology (LBC) method was developed, which is now applied equally to the conventional Pap smear. The implementation of

LBC technology in the Pap smear is one reason contributing to the decline in cervical cancer incidence (17-19).

This method differs from the conventional Pap smear because the cell sample is not immediately placed on a slide after collection but is instead placed in a liquid preservative to preserve and protect the cells from damage. Samples are processed in an automated device that uses centrifugal force to separate the cells from the liquid, then place the cells on slides, staining them for better visibility. Additionally, LBC slides are suitable for automated analysis (17, 20, 21).

With LBC, testing for HPV, gonorrhea, and chlamydia from a single sample is possible. This method has the advantages of easier interpretation, clearer backgrounds reducing the likelihood of epithelial cell occlusion, fewer unsatisfactory results, and filtering of blood and debris (17, 20).

The LBC technique involves collecting cells from the TZ using a Cervex-Brush, which is then transferred to a bottle with a liquid preservative. This brush has the advantage of allowing simultaneous sampling of cells from the TZ and endocervical region. However, a drawback of the Cervex-Brush is that it may cause epithelial damage and bleeding and is also expensive. There are two main sample preparation methods for LBC: SurePath and ThinPrep. The U.S. Food and Drug Administration (FDA) approved the use of SurePath in 1999, while it approved ThinPrep as a replacement for cervicovaginal smears in 1966. These methods differ in principle but produce similar preparations (16).

DIAGNOSTIC SENSITIVITY AND SPECIFICITY OF THE CONVENTIONAL PAP SMEAR METHOD AND LIQUID-BASED CYTOLOGY METHOD

In a study conducted in India, involving 100 randomly selected subjects, the conventional Pap smear test was found to be less effective compared to LBC. Samples had a higher percentage of satisfactory results with the LBC method. Furthermore, the sensitivity for LSIL and HSIL, as well as overall, was higher compared to the conventional method, and the specificity was also higher, except for LSIL detection (22).

Similarly, in a Japanese study involving 312 subjects, LBC was found to be a more sensitive method, while the conventional method was more specific. For CIN I, the specificity of the LBC method was lower, at 25% compared to 32.1% for the conventional Pap smear test. LBC had higher sensitivity and negative predictive value for detecting CIN I and CIN II, as well as overall (23).

According to research by Shobana et al. (22), the conventional method proved to be more specific in diagnosing LSIL and HSIL, with a specificity estimate of 93% compared to 49% and 100% compared to 96%, retrospectively. On the other hand, the LBC method showed higher sensitivity in diagnosing LSIL and HSIL, with a sensitivity estimate of 66% compared to 40% and 100% compared to 50%, respectively (22).

The results of prospective, prospective-observational, and cross-sectional studies have shown that overall, the LBC method is more diagnostically sensitive and specific compared to the conventional Pap smear test (22-25). Conversely, research conducted by Dhananjayaet al. (25) showed that the conventional method is more sensitive compared to the LBC method, with a sensitivity estimate of 33.33% compared to 22.22%, while the specificity of both methods was the same, at 96.65% (25).

FINDINGS OF THE CONVENTIONAL PAP SMEAR TEST AND LBC IN DETECTING CERVICAL ABNORMALITIES

The most common findings obtained by the LBC method are negative for intraepithelial lesion or malignancy (NILM), normal, and nonspecific inflammation, 46%, 21.5%, and 13.5%, retrospectively. The LBC method proved superior in detecting Candida spp., while the difference between these two methods was minimal regarding Trichomonas vaginalis, with only one additional case detected by the conventional Pap smear test. In this study, the diagnostic consistency was 83.9%. The LBC method showed better results in detecting endocervical, epithelial, and atrophic cells (66.7%, 25.4%, 88.5%, 85.5%, 7.4%, 3.8%, retrospectively). There was a slight difference in favor of the conventional Pap smear test in detecting metaplastic cells, with 1.1% compared to 0.8%. The LBC method stands out as significantly better in terms of false-negative diagnoses, with one case compared to 14 cases with the conventional Pap smear test. The LBC method can improve sample quality and reduce the number of unsatisfactory samples (26). A study conducted by Shobana et al. (22) revealed fewer abnormalities compared to the LBC method, 22% versus 28%, retrospectively (22).

In contrast, in a Pakistani population of 3.929 participants, the conventional method detected a higher number of Candida spp. cases, while a smaller number of Trichomonas vaginalis infections were identified. However, this may be due to the conventional method analyzing a larger number of samples, nearly 1.000 more. The LBC method detected a higher number of LSIL, HSIL, and

glandular epithelial lesions. For detecting NILM, the conventional Pap smear test performed better with 97.9% compared to 96.2% with LBC. The LBC method is cost-effective in mass screening for cervical cancer (27). In a study among the Indian population, the conventional Pap smear test was less effective compared to the LBC method, as a higher number of HSIL and squamous cell carcinoma cases were detected using the LBC method (28).

Among six studies investigating ASC-US (Atypical squamous cells of undetermined significance), the results showed similar trends. In three of these studies, the conventional method detected a higher number of cases (14.5% compared to 11.5%, 6% compared to 2.6%, 3.31% compared to 2%, retrospectively). In one study, both methods identified the same number of ASC-US cases, while differences in favor of the LBC method were minimal in the remaining studies, where only one additional case was recorded compared to the conventional method, and 1% compared to 0.6% in favor of the LBC method (23, 24, 27-30).

The results of three studies conducted in Japan, Thailand, and India, involving 312, 1206, and 97 participants, retrospectively, showed that the conventional method was better in diagnosing HSIL compared to LBC (23-25). Conversely, studies conducted in India, Pakistan, and Egypt, involving 200, 3929, and 150 participants, respectively, showed that the LBC method was better at diagnosing HSIL (27-29). It is important to note that studies supporting the LBC method included a larger number of participants, almost 2700 more.

According to a study by Ranjana et al. (30), the LBC method and the conventional Pap smear test show equal abilities in detecting the presence of LSIL and HSIL in younger participants (30).

QUALITY OF SAMPLE IN CONVENTIONAL PAP SMEAR AND LBC

A comparison of the quality and quantity of cervical tissue samples obtained by conventional Pap smear test and LBC method showed that components of TZ were present in 96.8% of samples by conventional Pap smear test and in 98.1% of LBC samples. Greater opacity was recorded with the conventional Pap smear test compared to LBC (24). LBC improves sample quality and reduces the likelihood of false-negative results, thus enhancing the effectiveness of screening programs (22).

One case of false-negative diagnosis was recorded with the LBC method, whereas with the conventional Pap smear test, there were 14 cases, highlighting the superiority of LBC in reducing the risk of false-negative diagnoses (26).

The conventional Pap smear test has an inadequacy rate ranging from 5% to 25% (31). Additionally, it has several shortcomings such as inadequate transfer of cells to slides, uneven cell distribution, and the presence of obscuring materials like inflamed cells, blood, and overlapping epithelial cells (32). LBC has the advantage of fewer unsatisfactory smears and fewer obscuring factors such as blood or mucus (28).

CONCLUSION

The LBC method stands out as a method with higher diagnostic sensitivity, especially in detecting LSIL, HSIL, and CIN. On the other hand, the conventional Pap smear test retains its specificity, especially in diagnosing LSIL. LBC demonstrates an advantage in terms of sample representativeness and a smaller number of unsatisfactory samples. This method provides greater reliability in diagnostic procedures, which is of exceptional importance for achieving high diagnostic accuracy and timely detection of potential abnormal changes. It is important to consider that the results of the study varied depending on the population, methodology, and sample size, suggesting the need for further research to confirm these findings and better understand the difference between these two methods.

Abbreviations
ASC-US - Atypical squamous cells of undetermined significance
CIN - Cervical intraepithelial neoplasia
FDA - U.S. Food and Drug Administration
HPV - Human papillomavirus
HSIL - high-grade squamous intraepithelial lesion
LBC - liquid-based cytology
LSIL - low-grade squamous intraepithelial lesion
SIL - squamous intraepithelial lesion
TZ - Transformation zone

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Sažetak

KOMPARACIJA KONVENCIONALNE METODE PAPA TESTA I TEČNE CITOLOGIJE U DETEKCIJI CERVIKALNIH ABNORMALNOSTI

Šečerović Đelila

Univerzitet u Sarajevu, Fakultet zdravstvenih studija, Laboratorijske tehnologije, Sarajevo, Bosna i Hercegovina

Rak cerviksa predstavlja jednu od najčešćih vrsta raka kod žena koji zahteva ranu dijagnostiku kako bi se smanjila prevalencija i smrtnost, a Papa test ima ključnu ulogu u ranoj dijagnostici prekanceroznih lezija. Konvencionalna metoda Papa testa se već dugo vremena koristi za rano otkrivanje lezija, dok se LBC metoda sve više koristi kao alternativna metoda sa potencijalnim prednostima.

U istraživanju, konvencionalni Papa test je pokazao manju efikasnost u poređenju sa LBC metodom. LBC metoda je imala veći postotak zadovoljavajućih uzoraka i pokazala je veću osetljivost i specifičnost za identifikaciju različitih abnormalnosti cerviksa. Slična otkrića su pronađena i u japanskom istraživanju. Međutim, postoje istraživanja koja su pokazala suprotne rezultate, naglašavajući specifičnost konvencionalne metode. Konvencionalna metoda Papa testa je u nekim istraživanjima pokazala veću sposobnost detekcije ASC-US-a, dok su u drugim istraživanjima rezultati bili slični ili u korist LBC metode.

LBC metoda se ističe po većoj dijagnostičkoj osetljivosti, posebno u otkrivanju različitih vrsta cervikalnih abnormalnosti, dok konvencionalna metoda Papa testa zadržava svoju specifičnost, posebno u dijagnostici LSIL-a. LBC metoda ima prednost zbog bolje reprezentativnosti uzoraka i manjeg broja nezadovoljavajućih uzoraka.

Ključne reči: rak grlića materice, Papa test, tečna citologija, prekancerozne lezije raka grlića materice, dijagnoza raka grlića materice, klasifikacija Papa testa.

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Correspondence to/Autor za korespondenciju

Šečerović Đelila
Pertac 26, Visoko 71 300, Bosnia and Herzegovina
phone:+387 61 224 123
email: delilaasecerovic@gmail.com
ORCID: 0009-0003-3081-8655