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International Clinical Pathology Journal

Review Article

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Modern approach to cervical cancer screening program–Georgian experience

Abstract

Objective: The study aimed to pilot the modern approach to cervical cancer screening program, which means the application of liquid based cytology and chromogenic in situ hybridization (CISH) for human papillomavirus (HPV) genotyping on atypical cervical smears.

Materials and methods: 1293 cervical cytology samples have been analyzed in country of Georgia. The samples had been collected and processed by the usage of materials and equipment provided by Hologic. Prepared smears were post-fixed in 96% ethanol and stained accordingly with Papanicolau protocol. The Bethesda 2001 system terminology was employed for reporting and diagnoses of cervical smears. Patients with diagnosed atypia were recalled for obtaining of material for HPV genotyping. This has been performed by usage of CISH method.

Results: The negative for intraepithelial lesion or malignancy (NILM) category was equal to 1156cases (89.40%). Other categories in decreasing order were atypical squamous cells of undetermined significance (ASCUS) with 104cases (8.04%), low grade squamous intraepithelial lesion (L-SIL) with 8cases (0.62%), high grade squamous intraepithelial lesion (H-SIL) with 1cases (0.08%), atypical squamous cells, cannot exclude high grade intraepithelial lesion (ASC-H) with 21case (1.63%) and atypical glandular cells of undetermined significance (AGUS) with 3case (0.23%). Cellularity was lower in liquid based cytology (LBC) as compared with conventional smears (CS). Also, nuclear overlap was significantly less observed compared to CS. The smear background was notably cleaner and cell morphology was better evaluated in LBC. In terms of Trichomonas and Candida detection, LBC was superior compared to CS. Doderlein lactobacilli were seen in significantly lesser amounts and were mainly situated in close vicinity to the squamous epithelial cells. Due to lack of pretreatment, the degree of inflammation was better assessed in CS.

Conclusion: Our experience shows that LBC is superior to CS in the evaluation of cell morphology and detection of certain microorganisms such as Trichomonas and Candida. The degree of inflammation is better assessed with CS. CISH is effective and easy for implementation method for HPV genotyping on cervical smears. There has been revealed 76.12% concordance in average between genotyping results and cytopathology findings of routine screening.

Keywords: liquid based cytology, conventional smear, cervical cancer, papanicolau, HPV, atypical squamous cell of undetermined significance, human papillomavirus

Volume 2 Issue 6 - 2016

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Received: July 24, 2016 | **Published:** September 23, 2016

Abbreviations: LBC, liquid based cytology; CS, conventional smear; NILM, negative for intraepithelial lesion of malignancy; SCUS, atypical squamous cell of undetermined significance; L-SIL, low grade squamous intraepithelial lesion; H-SIL, high grade squamous intraepithelial lesion; ASC-H, atypical squamous cell cannot exclude high grade intraepithelial lesion; AGUS, atypical glandular cells of undetermined significance; HPV, human papillomavirus; CISH, chromogenic in situ hybridization

Introduction

Cervical cancer is the severe health care problem. According with global statistics, cervical cancer is on the second place by the frequency and on the third by mortality among the cancers of reproductive system.[1–3] 527,000 newly diagnosed cases of cervical cancer and 265,000 deaths due to this health care problem were recorded in 2012 by World Health Organization. Most part (85%) of cervical cancer incidence and mortality occurred in developing countries,[2] those are characterized by the absence or ineffective and irregular screening programs.[2,3] The cytological screening is the main screening approach for cervical cancer. The Papanicolau stained conventional smear can be used for cervical cancer screening purposes, but some authors[2] complained on low diagnostical sensitivity because of false positive and false negative results. The amount of false-negatives varies from 2% to 50%.[1,4–6] In a meta-analysis study[7] the sensitivity of cervical cancer screening performed by application of conventional smear was declared as 58% (range 11%-99%), with a specificity of 68% (range 14%-97%).

Liquid-Based Cytology (LBC) method has been applied by Cytic Corporation (USA) for cervical cytology smears obtaining and collection in the 1990s. The method has been approved by the United States Food and Drug Administration in 1996 and introduced for cervical cancer screening as an alternative of the conventional smear.

LBC method enables suspension of the cells in liquid medium and preparation of cellular monolayer.[8–10] Nowadays two methodologies and solutions of LBC are widely available: ThinPrep (Hologic, Marlborough, MA, USA) and BD SurePath (BD Diagnostics–TriPath, Burlington, NC, USA).[11–15]

LBC is characterized by the improved sensitivity and specificity in comparison with conventional smear. The method is ensuring the better fixation and excellent preservation of nuclear details. Atypical cells are obvious, they aren't obscured by another cells or background. Furthermore, LBC method is characterized by the low rate of unsatisfactory samples. The application of LBC for cervical cancer screening in countries with middle and low income is limited due to the financial restrictions, conventional smear is still the basic method of cervical cancer screening in developing world.[16]

Nearly all sexually active men and women are exposed to human papillomavirus (HPV) at some stage in their lives; it does not cause health problems. HPVs that infect the anogenital tract fall into two broad groups: those that cause warts (low-risk) and those associated with cancer (high-risk). Persistent infection with high-risk HPV types causes all cervical cancer, most vulvar, vaginal and anal cancers, approximately half of penile cancers, as well as an increasing subset of oropharyngeal cancers, and HPV is also implicated on cancer precursor conditions in the cervix, anus, vulva and vagina. In some instances, HPV status will determine the approach to cancer treatment. The rising number of HPV-related cancers is a major public health issue. The concept of a virus causing cancer is frightening. The association of cervical precancer with HPV has clear psychosocial adverse effects. Health professionals must be prepared to discuss HPV status because affected patients may want to know the cause of their condition and may question the implications for their sexual partners. Although discussions about HPV between patients and health professionals are becoming more common in cervical disease, patients express concern about the stigma attached to sexual transmission. There is little rigorous research into how clinicians communicate with patients for the other cancers associated with HPV. Patients and the public know very little about HPV. Despite the introduction of HPV vaccination in schools, and HPV testing within cervical screening programmes in the UK and several other countries, systematic reviews demonstrate consistently poor knowledge and lack of awareness that HPV is a sexually transmitted causative factor for cervical cancer. Furthermore, women who are found to be HPV positive during cervical screening experience distress, anxiety and a notable lack of understanding. Health care professionals do not know enough about HPV-associated cancers, a developing area of research where there are still many uncertainties and good quality patient information is lacking.[17]

More than 200types of HPV have been recognized on the basis of DNA sequence data showing genomic differences. Based on their association with cervical cancer and precursor lesions, HPV is grouped to high-risk and low-risk HPV types. Low-risk HPV types include types 6, 11, 42, 43, and 44. High-risk HPV types include types 16, 18, 31, 33, 34, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, and 70.[18] It is recognized that persistent infection of the HPV is required for the development of invasive cervical cancer.[19] While infection with HPV is common, especially in sexually active young women, most infections are transient and are characterized by self-recurrence without clinical consequences. However, some women develop persistent HPV infections and are at risk for cervical cancer and its precursors. Cervical cancer is heralded as being the third most common cancer in women followed by breast and colorectal cancer. World Health Organization (WHO) has reported that approximately 530,000 women worldwide are diagnosed with cervical cancer, and the mortality of incidence ratio changed from 52 to 88% in developing countries. Although cervical cancer screening programs by cytology tests (e.g., conventional Pap-smears, LBS) has decreased the incidence and death rate in many countries in the past few decades, cervical cancer still remains a leading cause of death in women due to the high rate of false positive results are limited reproducibility of cytology tests. Besides, the sensitivity and specificity of the test have been questioned. The low sensitivity of cytology tests would put women at risk of developing invasive cervical cancer. Thus, much concern has arisen recently to develop a better screening test and/or design for disease prevention, especially the role of HPV testing.

Our aim was to pilot the modern approach to cervical cancer screening program, which means the application of liquid based cytology and chromogenic in situ hybridization (CISH) for human papillomavirus (HPV) genotyping on atypical cervical smears.

Materials and methods

1293 cervical cytology samples have been analyzed in country of Georgia. These were 18-65years old non vaccinated for HPV, gynecological asymptomatic females. The median age of screened group was 37years. Specific inclusion criteria have not been used for patients recruitment. Informed consent has been obtained for all cytology smears. All cases were taken by usage of the ThinPrep reagents (Hologic). The cervical smear was obtained by rover cervical brushes and washed in the sampling solution ThinPrep (Hologic). One package of sampling materials (cervical brush and vial with sampling solution ThinPrep) has been used per patient. After obtaining and before laboratory processing the samples were stored at room temperature. The delay time between obtaining of samples and their laboratory processing did not exceed 2hours. The smears have been prepared on glass slides by the application of the ThinPrep 2000 Processor (Hologic) accordingly with the provided for gynecology samples instructions, the program #4 of the processor has been used. One glass slide has been prepared for each screened patient. Prepared wet smears have been fixed in absolute alcohol during 30 min and stained accordingly with Papanicolau staining protocol (http://www.nottingham.ac.uk/pathology/protocols/papcytol.html). The Bethesda 2001 System terminology (http://nih.techriver.net/bethesdaTable.php) has been used for reporting of cervical smears. The average time required for processing and reporting of the sample was 4hours (92.7% of cases). The Papanicolau stained smears were evaluated by light microscopy (Konus, 5601-Biorex-2) under x4, x10, x40 and x100 objective lens. The stained smears have been archived accordingly with requirements to medical data storage and documentation specific to country of Georgia.

The re-usage of the rest of samples containing ThinPrep solution containing samples for additional smear preparation was impossible because of insufficient for smear preparation amount of liquid. Therefore, patients with diagnosed atypia were recalled within 5 working days (to avoid self-recurrence and/or new infection cases) for obtaining of material for HPV genotyping. The short time period has been elaborated to exclude and avoid situation of viral clearance and/or possible new infections. The HPV genotyping has been performed by CISH method on conventional smears. The smears have been fixed

Citation: Kldiashvili E, Bojgua S. Modern approach to cervical cancer screening program–Georgian experience. *Int Clin Pathol J.* 2016;2(6):139–143. DOI: 10.15406/icpjl.2016.02.00061

in absolute alcohol during 30 min. HPV specific DNA detection in the smear were performed by ZytoFast HPV type 16/18/31/33/35 Probe Kit (ZytoVision) and HPV genotyping by ZytoFast HPV type 16/18 and type 31/33 Probe Kits (ZytoVision) according to the manufacturer's instructions. Positive and negative controls were used as reference for the color appearance and reaction quality assurance.

Briefly, for enzyme digestion the smears were treated by pepsin solution on 37 ° C during 5minutes and then washed by distilled water at room temperature. For fixation smears were incubated in 1% formaldehyde solution (5 min, room temperature). After heat pretreatment step (incubation in EDTA for 15min, 98 ° C; wash distilled water for 1 min, room temperature) air dried smears were hybridized with dig-labeled probes (5µL, 5min at 75 ° C and 60min at 37 ° C). The detection of HPV DNA in smears was performed by rabbit-anti-DIG (30min at 37°C) and anti-rabbit-AP-polymer (30min, 37°C). For counterstain were used Mayer's hematoxylin (1min, room temperature). The stained smears were evaluated by light microscopy (Konus, 5601-Biorex-2) underx40 andx100 objective lens. Smears with stained red cells (AP-Permanent Red) on the blue background were considered positive for HPV DNA (16/18/31/33/35) and HPV concrete genotype (16/18, 31/33).

Results and discussion

One thousand two hundred ninety three cases were analyzed in our study. All cases were the cellular monolayer, nuclei overlap has not been seen. 1156 cases (89.40%) were reported as the negative for intraepithelial lesion or malignancy (NILM), atypical epithelial cells were seen in 134(10.37%) cases, and glandular cell atypia was reported in 3(0.23%) cases (Table 1). Among cases with reported abnormal cervical cytology the following reports were written: atypical squamous cells of undetermined significance (ASC-US)-104 cases (77.61%); atypical squamous cells, cannot exclude high grade squamous intraepithelial lesion (ASC-H)-21 cases (15.67%); low grade squamous intraepithelial lesion (LSIL)-8 cases (5.97%); high grade squamous intraepithelial lesion (HSIL)-1 case (0.75%). These results are given in Table 2.

HPV DNA has been revealed in 102cases (76.12%) from 134cases with diagnosed epithelial cell abnormalities. These cases were positive for high-risk HPV of 16/18/31/33/35 type. 16/18 types of HPV were revealed in 91cases (89.22%), 31/33 types of HPV were revealed in 11cases (10.78%). 32cases from 134cases (23.88%) with cytologically diagnosed atypia of epithelial cells, were negative for HPV DNA. All HPV negative cases were diagnosed as ASCUS during LBC screening. The correlation between HPV DNA detection and genotyping by application of 16/18 and 31/33 HPV types probes with LBC screening diagnosis are summarized in Table 3.

One thousand two hundred ninety three cases were analyzed in this study. It is obvious that LBC is effective and appropriate method for cervical cancer screening. Furthermore, the pilot study aimed standardization of reporting of cervical cancer screening results. Cancer is a top priority health care issue in country of Georgia. Cervical, breast, colorectal and prostate cancer screening programs are available in the country, but due to some psycho-social factors, limitations and barriers patients are attending the office of medical doctor only in the case of urgent necessity. As a result, more than half of all cancer cases are diagnosed in the late stages. It has been revealed, that different classification systems (e.g., the Bethesda System 2001, Papanicolau, Cervical Intraepithelial Neoplasia-CIN) are used in Georgia to communicate results of cytology tests. This is the most important factor of misunderstanding in the chain of medical service. It is obvious, that the cytology screening of cervical cancer is the effective screening test utilized in health care. It can be realized by application of conventional smear, or through application of LBC technology depending on the budget. It has been concluded, that the LBC based cervical cancer screening is more comfortable than conventional smear based one. Monolayer smears are easier for interpretation, cells with atypia are not obscured by other of cells or background (inflammation, blood and etc). Furthermore, the amount of unsatisfactory for interpretation smears is minimal, in the frames of our pilot we have not unsatisfactory samples. However, for LBC test to be effective, three things must occur:

i. Sampling should be adequate and proper.

ii. Sample processing, review and reporting should be proper and standardized.

iii. Reporting terminology should be standard and understandable for the clinician.

Regarding terminology it should be emphasized, that the most informative and adequate is the Bethesda 2001 System (TBS).[16] This is a comprehensive way to report cytologic peculiarities of the cervix by a simple diagnostic terms and the possibility to incorporate a descriptive diagnosis and evaluation of specimen adequacy.[20,21] Our experience shows that LBC is superior to CS in the evaluation of cell morphology and detection of certain microorganisms such as Trichomonas and Candida. The degree of inflammation is better assessed with CS. Furthermore, the HPV genotyping is very effective possibility to exclude the false positive cases of atypia.

Table 1 1156 cases (89.40%) were reported as the negative for intraepithelial lesion or malignancy (NILM), atypical epithelial cells were seen in 134(10.37%) cases, and glandular cell atypia was reported in 3(0.23%) cases

Category Number of Cases	Negative for intraepithelial lesion or malignancy	Atypical epithelial cells	Glandular cell atypia
Totally - 1293 Cases (%)	1156(89.4%)	134(10.37%)	3(15.53%)

Citation: Kldiashvili E, Bojgua S. Modern approach to cervical cancer screening program–Georgian experience. *Int Clin Pathol J.* 2016;2(6):139–143. DOI: 10.15406/icpjl.2016.02.00061

Table 2 Atypical squamous cells, cannot exclude high grade squamous intraepithelial lesion (ASC-H) - 21 cases (15.67%); low grade squamous intraepithelial lesion (LSIL) - 8 cases (5.97%); high grade squamous intraepithelial lesion (HSIL) - 1 case (0.75%)

Category Number of Cases	ASCUS	ASC-H	LSIL	HSIL
Totally - 134 Cases (%)	104 (77.61%)	21 (15.67%)	8 (5.97%)	1 (0.75%)

Table 3 HPV DNA has been revealed in 102 cases (76.12%) from 134 cases with diagnosed epithelial cell abnormalities. These cases were positive for high-risk HPV of 16/18/31/33/35 type. 16/18 types of HPV were revealed in 91 cases (89.22%), 31/33 types of HPV were revealed in 11 cases (10.78%)

CISH HPV	HPV DNA	HPV DNA	HPV DNA
LBC screening diagnosis	(16/18/31/33/35)	(16/18)	(31/33)
ASCUS	77 (75.49%)	71 (69.62%)	6 (5.88%)
ASC-H	14 (13.73%)	10 (9.8%)	4 (3.92%)
LSIL	7 (6.86%)	6 (5.88%)	1 (0.98%)
HSIL	4 (3.92%)	4 (3.92%)	--

Table abbreviations: ASCUS, atypical squamous cell of undetermined significance; L-SIL, low grade squamous intraepithelial lesion; H-SIL, high grade squamous intraepithelial lesion; ASC-H, atypical squamous cell, cannot exclude high grade intraepithelial lesion; HPV, human papillomavirus; CISH, chromogenic in situ hybrydization; DNA, deoxyribonucleic acid; LBC, liquid based cytology

Conclusion

The benefits of screening and early intervention are clear. Early intervention is available and can be performed in country of Georgia at minimal cost. There is no standardized approach for obtaining smears or interpreting results. The resulting ambiguity makes it difficult for clinicians to compare results of Pap-test, negatively affecting patient care. By the implementation of the present pilot the introduction of standardized and LBC approaches for cervical cancer screening has been performed. It has been revealed, that LBC improved sensitivity and specificity of cervical cancer screening since fixation is better and nuclear details are well-preserved, the amount of unsatisfactory samples is decreased. It has been also revealed, that CISH is effective and easy for implementation method for HPV DNA detection and genotyping on cervical smears. The efficacy of the high-risk HPV type (16/18/31/33/35) probe for detection of HPV infection has been confirmed. Furthermore, the prevalence of 16/18 type of HPV in atypical cases has been confirmed by the present pilot study. The 76.12% (HPV has been revealed in 102cases from totally atypical 134cases) concordance in average between genotyping results and cytopathology findings of routine screening has been revealed. 7.8% cases of atypical cases were HPV negative, all these cases were diagnosed as ASCUS during LBC screening. It has been concluded, that severe inflammation as well as specific inflammation (i.e., induced by Candida spp) can be misdiagnosed. Taking into account the atypical cases follow up guidelines, it has been concluded, that shift towards HPV DNA based cervical cancer screening will be effective and appropriate. The increased costs of cervical cancer screening will compensated by the higher specificity and prolonged screening intervals.

Acknowledgements

Provide list of individuals who contributed in the work and grant details.

The study has been performed during implementation of the project 'Cervical Cancer Screening Using Telemedicine Resources in Georgia' (#007G). This project was supported by 700 for Science and Hologic. The research has been performed by Ekaterina Kldiashvili, Ph.D. and Sophio Bojgua, Ph.D. Student in equal contribution.

Conflict of interest

No conflicts of interest in connection with the manuscript exist. No competing financial interests exist.

References

1. Costa MO, Heráclio SA, Coelho AV, et al. Comparison of conventional Papanicolaou cytology samples with liquid-based cervical cytology samples from women in Pernambuco, Brazil. *Braz J Med Biol Res*. 2015;48(9):831–838.
2. World Health Organization. *Comprehensive cervical cancer*. Geneva, Switzerland: WHO; 2014. 364 P.
3. World Health Organization. *Human papillomavirus (HPV) and cervical cancer*. Geneva, Switzerland: WHO; 2016.
4. Schiffman M, Solomon D. Findings to date from the ASCUS-LSIL Triage Study (ALTS). *Arch Pathol Lab Med*. 2003;127(8):946–949.
5. Bergeron C, Masseroli M, Ghezi A, et al. Quality control of cervical cytology in high-risk women. PAPNET system compared with manual rescreening. *Acta Cytol*. 2000;44(2):151–157.

6. Fahey MT, Irwig L, Macaskill P. Meta-analysis of Pap test accuracy. *Am J Epidemiol*. 1995;141(7):680–689.
7. Gerhard R, Schmitt FC. Liquid-based cytology in fine-needle aspiration of breast lesions: a review. *Acta Cytol*. 2014;58(6):533–542.
8. Takei H, Ruiz B, Hicks J. Cervicovaginal flora. Comparison of conventional pap smears and a liquid-based thin-layer preparation. *Am J Clin Pathol*. 2006;125(6):855–859.
9. Stabile SAB, Evangelista DHR, Talamonte VH, et al. Comparative study of the results from conventional cervico-vaginal oncotic cytology and liquid-based cytology. *Einstein*. 2012;10(4):466–472.
10. Bidus MA, Maxwell GL, Kulasingam S, et al. Cost-effectiveness analysis of liquid-based cytology and human papillomavirus testing in cervical cancer screening. *Obstet Gynecol*. 2006;107(5):997–1005.
11. Ronco G, Segnan N, Giorgi-Rossi P, et al. Human papillomavirus testing and liquid-based cytology: results at recruitment from the new technologies for cervical cancer randomized controlled trial. *J Natl Cancer Inst*. 2006;98(11):765–774.
12. Abulafia O, Pezzullo JC, Sherer DM. Performance of ThinPrep liquid-based cervical cytology in comparison with conventionally prepared Papanicolaou smears: a quantitative survey. *Gynecol Oncol*. 2003;90(1):137–144.
13. Arbyn M, Herbert A, Schenck U, et al. European guidelines for quality assurance in cervical cancer screening: recommendations for collecting samples for conventional and liquid-based cytology. *Cytopathology*. 2007;18(3):133–139.
14. Hoelund B. Implementation of liquid-based cytology in the screening programme against cervical cancer in the County of Funen, Denmark, and status for the first year. *Cytopathology*. 2003;14(5):269–274.
15. Nandini NM, Nandish SM, Pallavi P, et al. Manual liquid based cytology in primary screening for cervical cancer--a cost effective preposition for scarce resource settings. *Asian Pac J Cancer Prev*. 2012;13(8):3645–3651.
16. Solomon D, Nayar R. *The Bethesda System for Reporting Cervical Cytology*. USA: Springer; 2004.
17. Hendry M, Pasterfield D, Adams R, et al. Talking about human papillomavirus and cancer: protocol for a patient-centred study to develop scripted consultations. *BMJ Open*. 2016;69(4):e011205.
18. Burd EM. Human papillomavirus and cervical cancer. *Clinical Microbiol Revs*. 2003;16(1):1–17.
19. Walboomers JM, Jacobs MV, Manos MM, et al. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. *J Pathol*. 1999;189(1):12–19.
20. Katz IT, Wright AA. Preventing cancer in developing world. *N Engl J Med*. 2006;354(11):1110.
21. Debby L, Sankaranarayanan R. Secondary prevention of cervical cancer. *Int J Gynecology & Obstetrics*. 2006;94(Suppl1):S65–S70.
22. World Health Organization. *Comprehensive Cervical Cancer Control: A Guide to Essential Practice*. Geneva, Switzerland: WHO; 2013. p. 1–408.
23. Kavatkar AN, Nagwanshi CA, Dabak SM. Study of manual method of liquid based cervical cytology. *Indian J Pathol Microbiol*. 2008;51(2):190–194.
24. Hoda RS. Non-gynecologic cytology on liquid-based preparations: A morphologic review of facts and artifacts. *Diagn Cytopathol*. 2007;35(10):621–634.
25. Ronco G, Cuzick J, Pierotti P, et al. Accuracy of liquid based versus conventional cytology: Overall results of new technologies for cervical cancer screening: Randomized controlled trial. *BMJ*. 2007;335(7609):28.

Citation: Kldiashvili E, Bojgua S. Modern approach to cervical cancer screening program–Georgian experience. *Int Clin Pathol J.* 2016;2(6):139–143.
DOI: 10.15406/icpjl.2016.02.00061