Authors:

Ekaterina Kldiashvili, Ph.D., Executive Director, Georgian Telemedicine Union (Association), Tbilisi, Georgia

Fisnik Kurshumliu, MD.PhD., Institute of Anatomic Pathology at the Medical School in Pristina University; American Hospital Kosova

Title: **Application of liquid based cytology for cervical cancer screening – experience from Georgia and Kosovo**

Corresponding author:
Ekaterina Kldiashvili, Ph.D.
Executive Director
Georgian Telemedicine Union (Association)
75 Kostava str., 0171 Tbilisi, Georgia
Email: kldiashvili@georgia.telepathology.org

Fisnik Kurshumliu, MD.PhD.
American Hospital Kosova, Hajvali, Km7, Pristina, Kosovo
e-mail: fisnik.kurshumliu@spitaliamerikan.com

Abstract

Objective: The study aimed to evaluate the feasibility of liquid based cytology, namely ThinPrep as a tool for cervical cancer screening in Georgia and Kosovo.

Materials and Methods: 1500 and 703 cervical cytology samples had been analyzed in two Institutions in Georgia and Kosovo, respectively. The samples had been processed by the ThinPrep 2000, post-fixed in 96% ethanol and stained with Papanicolau method. The Bethesda 2001 system was employed for reporting.

Results: In cases from Georgia, the NILM category was the most prevalent, accounting for 1323 cases (88.2%). Other categories in decreasing order of frequency were: ASCUS with 127 cases (8.47%), L-SIL with 13 cases (0.87%), H-SIL with 6 cases (0.4%), ASC-H with 26 cases (1.73%), AGUS with 3 cases (0.2%), AEC with 2 cases (0.13%).Similar situation was recorded in Kosovo. The NILM category was equal to 652 cases (92.75%). Other categories in decreasing order of frequency were ASCUS with 42 cases (5.97%), L-SIL with 6 cases (0.85%), H-SIL with 2 cases (0.28%) and ASC-H with 1 case (0.14%). Cellularity was lower in LBC as compared with conventional smears. Also, nuclar 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 assesed in CS.

Conclusion: We did not observe any real logistical, technical and professional obstacles for the use of ThinPrep methodology in cervical cancer screening. The only possible negative implications are the cost, especially when it is done in private settings.

Key words: Liquid Based Cytology, Conventional Smear, Pap smear, Cervical Cancer

Introduction

Squamous cell carcinoma of the uterine cervix is the second most frequent cancer type and the third leading cause of cancer related mortality in women worldwide (1–3). According to World Health Organization 527,000 new cases and 265,000 cervical cancer related deaths were recorded in 2012. Eighty five percent of these occured in developing countries (2). Absence or low effectiveness of prevention programs are the main causes of cervical cancer related morbidity and mortality in these countries (2,3). The main prevention tool against cervical cancer is cytological screening and vaccination against oncogenic Human Papilloma Virus (O-HPV) types (2,3). The conventional Pap smear (CP) is considered an efficient and easy-to-apply methodology, as it has the ability to identify precursor lesions of cervical cancer while they are still treatable (2). In spite of effectivity of screening with Pap smear, the sensitivity of this method is still hampered by false positive and false negative interpretations (4). Rates of false-negatives vary from 2% to 50% (1,4–6). In a meta-analysis study conducted by Fahey et al. (7), sensitivity of the CP was found to be 58% (ranging from 11% to 99%), with a specificity of 68% (ranging from 14% to 97%).

In the 1990s, a liquid-based cytology (LBC) method was developed for collection and preparation of cervical cytology samples, namely ThinPrep (Cytyc Corporation, USA). Approved in 1996 by the United States Food and Drug Administration, ThinPrep was introduced as an alternative to using the conventional method, with the purpose of improving the identification of atypical cells. LBC is a technique that enables cells to be suspended in liquid medium and subsequently spread in a monolayer for better morphological assessment. It includes the preparation and evaluation of cells collected in a liquid fixative. It is employed to improve the sensitivity of the Pap test. The monolayer of cells facilitates interpreation of abnormal cells by the cytopathologist. In fact, liquid based preparations are increasingly being used for both gynecologic and non-gynecologic cytology, including fine needle aspirations (8). Two technologies, ThinPrep (Hologic, Marlborough, MA, USA) and BD SurePath (BD Diagnostics – TriPath, Burlington, NC, USA) are more widely used.

The advantages of LBC include improved sensitivity and specificity since fixation is better and nuclear details are well-preserved. Abnormal cells are not obscured by other epithelial or inflammatory cells or debris. There is, therefore, a lower rate of unsatisfactory cytology samples.

The residual cell suspension can be used to carry out further cytological preparations or other molecular tests such as detection of oncogenic Human Papilloma Virus (O-HPV) (1,9–13). Immunocytochemistry for p16INK4a may also be performed on the residual sample (4). This technique has been widely adopted and is gradually replacing the CP in cervical cancer screening programs in some countries (14,15)

There are certain financial and infrastructural implications when it comes to the routine use of LBC in developing countries. Manual methods as cost effective means for low resource settings have been also developed (16).

As it was the case that ThinPrep was introduced in Georgia and Kosovo at the same time, the aims of this study were to evaluate the feasibility of ThinPrep as a methodology in terms of ease of installation, procedure, interpretation and cost in these two countries in paralel.

Materials and methods

During the period of 14 months, 1500 and 703 cervical cytology samples had been analyzed in two central laboratories in Georgia and Kosovo, respectively. The study subjects were 18 to 65 years old asymptomatic women with various cultural and social status. The samples had been obtained by cytobrush collecting device and placed in the ThinPrep vials with corresponding fixative (Hologic, Marlborough, MA, USA). The samples had been stored at room temperatore and referred to the central laboratory at one- and two-weeks intervals in Pristina and Tbilisi, respectively. Samples were processed in ThinPrep 2000 (Hologic, Marlborough, MA, USA) according to manufacturer's instructions, post-fixed in 96% ethanol and stained with Papanicolau method. The Bethesda 2001 system was employed for reporting. Previously, the cytologist and pathologist who were appointed to read the slides, had a one week training at Hologic's facility in Rome – Italy, in order to get the necessary insights regarding the possible slight differences between conventional and LBC slide. Patient data such as: age, place of residence, ethnicity, educational level, profession, marital status, parity, age at first sexual intercourse, last gynecological check up and last Pap smear were recorded in a data base. Writen consent was obtained from all the study subjects.

Results

A total of 1500 and 703 cervical cytology samples were analyzed over a period of two years at two central laboratories in Tbilisi and Pristina, respectively.

NILM category was the most prevalent in Georgian samples, accounting for 1323 cases (88.2%). Other categories in decreasing order of frequency were: ASCUS with 127 cases (8.47%), L-SIL with 13 cases (0.87%), H-SIL with 6 cases (0.4%), ASC-H with 26 cases (1.73%), AGUS with 3 cases (0.2%), AEC with 2 cases (0.13%) (Table 1).

Similar situation was recorded in Kosovo (Table 2). The NILM category was equal to 652 cases (92.75%). Other categories in decreasing order of frequency were ASCUS with 42 cases (5.97%), L-SIL with 6 cases (0.85%), H-SIL with 2 cases (0.28%) and ASC-H with 1 case (0.14%).

Cellularity was lower in LBC as compared with conventional smears. Also, nuclar 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 assesed in CS.

Discussion

The conventional Pap smear (CP) is considered an efficient and easy-to-apply methodology, as it has the ability to identify precursor lesions of cervical cancer while they are still treatable (2). In spite of effectivity of screening with CP, the sensitivity of this method is still hampered by false positive and false negative interpretations (4). Rates of false-negatives vary from 2% to 50% in different studies (1,4–6).

In the 1990s, a liquid-based cytology (LBC) method was developed for the purpose of improving identification of atypical cells. LBC is a technique that enables cells to be suspended in liquid medium and subsequently applied to a microscope slide as a monolayer for better morphological assessment. This monolayer facilitates interpreation of abnormal cells by the cytopathologist. In

fact, liquid based preparations are increasingly being used also for non-gynecologic cytology, including fine needle aspirations (8). Two technologies, ThinPrep (Hologic, Marlborough, MA, USA) and BD SurePath (BD Diagnostics – TriPath, Burlington, NC, USA) are more widely used. The advantages of LBC include improved sensitivity and specificity since fixation is better and nuclear details are well-preserved. Abnormal cells are not obscured by other epithelial or inflammatory cells or debris. There is, therefore, a lower rate of unsatisfactory cervical cytology samples. The residual cell suspension can be used to carry out further cytological preparations or other molecular tests such as detection of oncogenic Human Papilloma Virus (O-HPV) (1,9–13). Immunocytochemistry for p16INK4a may also be performed on the residual sample (4).

As it was the case that ThinPrep was introduced in Georgia and Kosovo at the same time, the aims of this study were to evaluate the feasibility of ThinPrep methodology in terms of ease of installation, procedure, interpretation and cost in these two different countries. During the period of 2014 to 2015, 1500 and 703 women of different social, cultural and economic background were screened in Georgia and Kosovo, respectively.

Of course, there are certain infrastructural and financial implications when it comes to the routine use of LBC in developing countries.

At current times, the vast majority of the Gynecologists are used to conventionally collecting the sample with a spatula and spread it to the microscope glass which is subsequently either sprayed with or dipped in 96% ethanol solution. On the other hand, the proportion of cases that are reported unsatisfactory for evaluation by the cytologist/pathologist, is unrealistically low, compromizing the overall sensitivity and the drive for newer alternatives.

Instalation and operation of the ThinPrep 2000 in Kosovo was rather straitforward and took place at a laboratory within the American Hospital Kosova. One laboratory technician had a brief hands-on training on operation and maintanance of the device. As the Gynecologists had sufficient knowledge on how to use a cytobrush there were no difficulties in terms of sample collection. In purpose, we placed the device in one of the bigest private hospitals in Kosovo in order to keep the whole procedure, from sample collection to intepretation, under control. There was only one technician who prepared the samples, three Gynecologists who carried out sample collection and one Pathologist who reviewed the slides. This way, the number of cases that were unsatifsfactory for evaluation, was lower than 1%. Installation and operation of the ThinPrep 2000 in Tbilisi, Georgia took place at a laboratory within the New Vision University Hospital.

One laboratory assistant carried out the instalation, operation and maintanance of the device. Gynecologist had sufficient professional skills on the usage of a cytobrush as she had been previously trained.There were no difficulties in terms of sample collection. The whole process of sample collection, processing and interpretation was carried out by one laboratory assistant, one gynecologist and one clinical cytologist. The number of unsatisfactory for evaluation cases was lower than 1%."

After merging data from Georgia and Kosovo we realized that the prevalence of abnormalities in the two countries was similar even though the total number of the screened women in Georgia was twofold compared to Kosovo.

Cancer is an emerging public health issue in Georgia and Kosovo. The leading cause of cancer related moratlity in Georgian and Kosovan women are breast, uterine cervix and ovary. Unfortunately, a siginificant number of cases are diagnosed in late stages, missing the opportunity for effective treatment.

According to the literature, the benefits of screening women younger than 21 years are small because of the low prevalence of lesions that will progress to invasive cancer. Screening is also not beneficial in women older than 65 years if they have had a history of recent negative tests (17–19).

According to a survey by the US National Center for Health Statistics, based on an intervew of 16.467 women aged 21 and older, who denied any history of cancer, overall 20% of paticipants reported having had at least one abnormal Pap smear (20). In our study, the number of abnormal Pap smears was lower, namely 11.8% in Georgia and 7.24 in Kosovo. The reported ASCUS rate in Georgia and Kosovo was 8.47% and 5.97 respectively, whereas that reported in the literature is in the range of 2-10% (21–23). On the other hand the ASC/SIL ratio was 8 and 5 in Georgia and Kosovo, respectively. These figures are high compared to the recommendations reported in the literature and is an issue that should be addressed in both laboratories (24). However, the Bethesda terminology for interpreation of cervical cytology is rather recent in both countries and further efforts need to be made in order to use this classification in a wide scale.

In conclusion, instalation and operation of TinPrep 2000 was very straitforward in both settings in Georgia and Kosovo, respectively. Storage of ThinPrep vials for sample collection, pre- and post procedure required some additional laboratory space, however. Gynecologists reported that cytobrush was much superior for sample collection both in terms of ease of use and confidence

for better sampling, compared to spatula, which is generaly more commonly used in Georgia and Kosovo. The number of cases that were rendered unsatisfactory for evaluation was lower than 1% and these were all due to low cellularity of the sample. In terms of sample reading, the cytologist and pathologist reported a higher confidence for cell morphology evaluation. Certain microorganisms, such as Candida and Trichomonas were more reliably identified in ThinPrep. Doderlein lactobacilli however, were less in numbers and mainly situated in close vicinity to the epithelial cells. As the inflammatory background was cleared in ThinPrep, it was difficult to get an idea on the inflammatory component and it's impact on the epithelial cell morphology.

In summary, we did not observe any real logistical, technical and professional obstacles for the use of ThinPrep methodology in cervical cancer screening. The only possible negative implications are the cost, especially when it is done in private settings. ASC/SIL ratio was rather high compared to recommendations and is an issue that needs to be addressed in future screening.

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Disclosure/Conflict of Interest

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

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