The level of inadequate Pap smears collected by the Gynecology Service of Fier Regional Hospital in Albania

Eduard KAPAJ, MD
Obstetrics and Gynecology Service,
Fier Regional Hospital, Fier, Albania
Corresponding author: E-mail:edikapaj@yahoo.com

Ervin TOÇI, MD, Dr. Sc
Department of Public Health, Faculty of Medicine,
University of Medicine, Tirana, Albania

Sonila KAPAJ, MD
Obstetrics and Gynecology Service, Fier Regional Hospital,
Fier, Albania

Arben MITRUSHI, MD, Prof. Asoc. Dr.
Department of Morphology, Faculty of Medicine,
University of Medicine, Tirana, Albania

Abstract

Purpose: *This study aimed to determine the level of smears inadequate for Pap test in a population of women in Fier Prefecture in Albania.*

Material and methods: *This is a cross-sectional study including 1254 women who showed up for a Pap test at the gynecology service at the Fier Regional Hospital during 2013 and 2014. The women presented to our service either on their own initiative to perform the Pap test or recommended by the family doctor for this examination.*

Standard criteria were applied to classify a cervical smear as inadequate for interpretation. Reasons for the inadequate taking of Pap smears are discussed.

Results: *A total of 234 patients (96.2% females) were included. The average age of women in our study was 39.8 years ± 10.7 (34.2% were 31-40 years old and 27.1% were 41-50 years old). Overall, 9.6% of the cervical smears they were taken inadequately (there was a need to repeat the obtaining of the smear). Almost all materials were obtained from the exocervix and endocervix (in 98.1% of cases for which there is information on this variable). The absence of endocervical cells was the most frequent reason for classifying a smear as unsuitable for interpretation. Insufficient equipment, scarce material for cytological analysis and poor technical preparation can lead to the non-detection of abnormalities and errors in the microscopic analysis of smears.*

Conclusions: *Each member of the team is responsible for the accuracy of the results, but also for the adequacy of the sample. Inadequate smears could lead to the need for repeat tests and increased health care costs. Regular internal control, work quality monitoring as well as continuous training of all experts involved in obtaining, storing and interpreting cervical smears could reduce the level of inadequate Pap smears.*

Keywords**:** *Albania, adequacy, cervical cancer, cervical smear, Pap test.*

Introduction

The Pap test is one of the most effective screening tests for a common cancer, such as cervical cancer. Its massive use in developed countries has made squamous cell carcinoma the 10th most common cancer in women, while in developing countries where screening programs are not consolidated or not offered, this carcinoma is the most frequent malignant lesion among women (Parkin, Läärä and Muir, 1988). Clearly, the application of the Pap test for the screening of cervical cancer has been accompanied by a spectacular decrease in the incidence of this cancer in all developed countries, as for example in Iceland, the Nordic countries, Canada, the USA, etc. (Gustafsson et al., 1997). Meanwhile, in Norway, which decided not to implement a national screening program based on the Pap test, an increase in the incidence of squamous cell carcinoma of the cervix was observed between 1955 and 1975; subsequently, with the introduction of Pap test screening, a large reduction in the incidence of squamous cell carcinoma of the cervix was observed (Lönnberg et al., 2015). These data demonstrate the effectiveness of Pap test-based screening in reducing the incidence of squamous cell carcinoma in human populations; most likely the Pap test is the only effective screening test for cancer to date (Lieu, 1996; De Strooper et al., 2016).

An ideal screening test should have 100% sensitivity, however, few screening tests are 100% sensitive and the Pap test is no exception. A recent study conducted by the College of American Pathologists reported a Pap test false-negativity rate ranging from 5% (if a positive sample is defined as LSIL or worse) to 12.5% (if a positive sample is defined as ASCUS or worse); these errors refer only to false-negative results of laboratory origin, not including false-negative results due to error in obtaining or storing tissue samples (Lieu, 1996).

Intra-observer variation, such as over-diagnosis and under-diagnosis, is the main cause of misdiagnosis and occurs in all observers, regardless of the number of years of experience performing cytological examinations; interobserver variation also contributes to misdiagnosis, although this is influenced by the number of years of experience; sampling error (incorrect procedures for obtaining vaginal or cervical tissue material or in storing the material) in both cytological examination and biopsy contributes to discrepancies between cytological and histological diagnosis (Lieu, 1996).

The issue of inappropriate smears is important as it does not allow reading the smear and interpreting the results. This can lead to the loss of precious time for women, delaying the diagnosis and possible treatment of cervical lesions. An inadequate Pap test, by definition, is one in which the detection of abnormalities of the cervical epithelium is impossible and uncertain; for this reason, the ability to detect mild and severe intraepithelial lesions is significantly reduced and false negative diagnoses are also possible [Gavranović, Novak and Bolanca, 2011]. For a smear to be considered suitable for the Pap test, at least 10% of well-preserved and visible squamous cells must be present, being suitable for cytological analysis, according to the Bethesda Classification System (Solomon et al., 2002).

In our country, the Pap test continues to be the main screening test for cervical lesions. However, data on the level of inappropriate smears is lacking. In this context, the aim of this study was to determine the level of smears inadequate for Pap test in a population of women in Fier County, as well as to shed light on the factors that influence this phenomenon.

Methodology

Study design

This is a cross-sectional study. The study population included all women who showed up for a Pap test at the gynecology service at the Fier Regional Hospital during 2013 and 2014.

In total, during this period, 1254 women presented to our service either on

their own initiative to perform the Pap test or recommended by the family doctor for this examination.

Data collection

Initially, women's basic socio-demographic data were collected, such as age, place of residence, marital status.

In addition, in the framework of this scientific study, other data were collected, which are mainly related to the taking of smears in participating women. The obtained material was evaluated for its adequacy: if the examination of the smear showed that there was not a sufficient number of cells, or the cells were clustered, or they were obscured blood, inflammatory processes or mucus, then this sample was considered inappropriate. Samples obscured to the level of more than 75% by blood or inflammation were considered unsuitable for the Pap test.

To evaluate a sample obtained from the cervix as adequate, we applied the criteria of the Bethesda System, according to which an adequate sample for Pap test must contain two groups of five endocervical cells and/or squamous metaplastic cells; at least ten percent of the lamella should be covered with cellular material. Once the sample is assessed as satisfactory, elements such as the presence or absence of a transformation zone, or obscuration from blood or inflammation can be reported (Pangarkar, 2022). Regarding the presence of cells, in smears adequate for Pap test they should range between 8,000 and 20,000, with a minimum limit of 5,000 cells but which can go up to 2 thousand in atrophic smears, after hysterectomy or after therapy; relative to the transformation zone, an adequate smear should have at least ten well-preserved endocervical or squamous metaplastic cells that are solitary or organized in groups (Pangarkar, 2022).

Statistical analysis

Absolute numbers and corresponding percentages were used to describe categorical data. All statistical analyzes were performed through the statistical package Statistical Package for Social Sciences, version 26 (IBM SPSS Statistics for Windows, version 26).

Results

Table 1 presents data related to the distribution of women in the study according to their age group. The average age of women in our study was 39.8 years. The most frequent age group was 31-40 years old, represented by 34.2% of women, followed

by the 41-50 age group (27.1%), the 21-30 age group (19.5%) and the age group >50 years represented by 16.4%. Only 2.7% of the subjects were 20 years of age or younger at the time of the study.

TABLE 1. Distribution of subjects in the study according to age group

Variable	Absolute number	Percentage (%)
Total	1254	100.0
Age (mean ± standard deviation)	39.8 ± 10.7	
Age-group ≤20 years 21-30 years 31-40 years 41-50 years >50 years	 34 245 429 340 206	 2.7 19.5 34.2 27.1 16.4

Table 2 presents the data related to the adequacy of the smears obtained for cytological examination. It turned out that in 90.4% of cases the smears for the cytological examination were taken adequately, while in 9.6% of the cases they were taken inadequately (there is a need to repeat the obtaining of the smear).

TABLE 2. Data related to the adequacy of obtaining the Pap smear

Variable	Absolute number	Percentage (%)
Adequacy of Pap smear Yes No	 1134 120	 90.4 9.6
Total	1254	100.0

Table 3 presents data related to the type of material used for cytological examination, referring to the anatomical region from which the material was obtained.

Almost all materials were obtained from the exocervix and endocervix (in 98.1% of cases for which there is information on this variable). Meanwhile, in 1.5% of cases the material was taken only from the exocervix, in 0.2% of the cases it was taken from the endocervix, in 0.2% of the cases it was taken from the vagina + exocervix and in 0.1% of the cases it was taken from the vagina + exocervix + endocervix (Table 3).

TABLE 3. Data related to the type of material used for cytological examination

Variable	Absolute number	Percentage (%)	Valid percentage (%)
Material type			
From the exocervix	19	1.5	1.5
From the endocervix	2	0.2	0.2
From exocervix + endocervix	1217	97.0	98.1
From the vagina + exocervix	2	0.2	0.2
From the vagina + exocervix + endocervix	1	0.1	0.1
Missing da	13	1.0	
Total	1254	100.0	100.0

Discussion

The current study is one of the few studies that sheds light on the level of smears that are inadequate for the Pap test examination in our country. This study reported that about one-tenth (9.6%) of the smears were taken in inadequate ways.

Our results are much higher compared to a study carried out in Tirana, which refers to 5416 smears tested between the period January 2009 - January 2012 at the Obstetric-Gynecological University Hospital "Queen Geraldine" (Xhani and Filipi, 2013). The average age of women in this study was 42.8 years (Xhani and Filipi, 2013), while in our study the average age of women was around 40 years. In the study in Tirana, about 99.1% of smears were considered suitable for Pap test, while 0.9% of smears were considered unsuitable based on the Bethesda System (Xhani and Filipi, 2013). For comparison, in our study in the Fier District, about 9.6% of smears were categorized as inadequate, a much higher level compared to the result in the Tirana study. It is unclear the reason for this large difference in the level of strips unsuitable for interpretation between our study and the study in Tirana. It is necessary to carry out other studies to replicate our findings, or those of the study in Tirana, and illuminate the reasons for this discrepancy.

However, this level of unsuitable smears in Fier County is comparable to a study in Croatia, where among 12,242 smears, 1,594 or about 13% of them were considered unsuitable for interpretation (Gavranović, Novak and Bolanca, 2011).

The reasons for this high level of unsuitable smears in our study could be many. Some reasons for considering smears inappropriate include obscuring of the smear from the presence of red blood cells or inflammation. One reason may be related to the time when the cervical sample was taken (taking cervical material during a woman's period, for example). Also, the presence of inflammation is something common in the cervical material that will be subjected to the Pap test (Baka et al., 2013), and the presence of inflammatory changes in the Pap test is not an absolute indicator of genital tract infection, especially in asymptomatic women given that

a significant percentage of women with inflammation in the Pap test (59.6%) and of women with no inflammation in the Pap test (32.9%) are positive for various pathogens in the cervical culture (Baka et al., 2013). If the cytological criteria of the presence of inflammation in the Pap test are met, then a note is usually made to take this detail into account; however, the presence of inflammation in the Pap test is usually associated with a low predictive value regarding the presence of genital pathogens, especially in asymptomatic women (Bertolino et al., 1992).

The most common reasons for an inadequate sample are the lack of endocervical epithelium, the density of staining, and the coverage of cells with numerous inflammatory elements and erythrocytes; other reasons are related to the presence of foreign material, poor cell fixation or poorly stained preparations (Gavranović, Novak and Bolanca, 2011).

The presence of endocervical cylindrical cells and metaplastic cells is evidence that the sample was taken correctly; the smears that we consider satisfactory must be taken from a suitable anatomical site, with a sufficient number of cells; the material should be well fixed as well as suitably colored.

In our results, the absence of endocervical cells was the most frequent reason for classifying a smear as unsuitable for interpretation. The absence of endocervical cylindrical cells and especially metaplastic epithelial cells from the transformation zone leads to the possibility of a false negative test result, because the sample is not representative of the anatomical site.

In a smear that is not well taken, the cells can be damaged or applied in a thick layer (thicker than necessary). In these cases, it is impossible to assess the abnormality due to the covering of the cells either by inflammatory elements or blood. Therefore, before taking the cell sample, it is necessary to wipe the cervix with cotton to remove the layer of exfoliated, dead cells and excess mucus; likewise, when applying the material to the slide, care must be taken to place the cells with a sliding movement so as not to damage them and they must be placed in a thin layer; cells that are placed in a thick layer tend to fix poorly, so the color of the preparation is weak; if the material is not well fixed, the cells cannot be stained correctly; dried cells do not take ink well and such a smear cannot be read.

Staining with hematoxylin for a long time leads to more intense staining of the nuclei, which results in hyperchromasia of the nuclei; the chromatin is dark, the visualization of the chromatin distribution is poor and it is possible to evaluate such smear as abnormal (Sahay et al., 2013); unfiltered hematoxylin can also hinder qualitative analysis of preparations; very strong staining with cytoplasmic dyes "orange G (OG)" and "eosin azure (EA)" can also make analysis of the preparation difficult (Ethos Biosciences, 2022).

The introduction of the Bethesda classification system led to an increase in the accuracy of the diagnosis, mainly due to the appropriateness of the sample (Islam

et al., 2004). Based on literature data, it is estimated that the level of inappropriate smears fluctuates between 3.% and 5.9% (Islam et al., 2004; Treacy et al., 2009). A study in India among 1650 women reported a rate of 6.4% of smears unsuitable for interpretation (Sachan et al., 2018). In developed countries, the level of inappropriate smears is very low. For example, a study among 56,563 Pap test smears reported that the rate of smears classified as inappropriate was only 0.47% (Quiroga-Garza et al., 2014). However, as we mentioned above, in our study this level was 9.6%, i.e. higher than the reports in the literature, but we recall that an even higher level than ours was reported in Croatia where 12.8% of smears were considered unsuitable for interpretation (Gavranović, Novak and Bolanca, 2011). In this context, we can state that the result related to the level of cervical smears taken inappropriately in Fier Prefecture is within the levels reported in the international literature.

Researchers have shown that the samples are more adequate if the endocervical smear is obtained with adequate equipment and recommend the use of the "broom-type" brush, which allows for the simultaneous collection of elements from the endocervix and exocervix (Day, Deszo and Freund, 2002). As a reminder, about 98.1% of the materials obtained in our study refer exactly to the area of the endocervix and the exocervix simultaneously.

Conclusion

Insufficient equipment, scarce material for cytological analysis and poor technical preparation can lead to the non-detection of abnormalities and errors in the microscopic analysis of smears; this means that each member of the team is responsible for the accuracy of the results, but also for the adequacy of the sample; it is possible for the above causes to be reduced or minimized if regular internal control is carried out, work quality monitoring as well as continuous training of all experts involved in obtaining, storing and interpreting cervical smears. Finally, if cervical epithelial abnormalities or lesions are not adequately detected, this may lead to the need for repeat tests, automatically leading to increased health care costs.

References

Baka S, Tsirmpa I, Chasiakou A, et al. Inflammation on the cervical papanicolaou smear: evidence for infection in asymptomatic women?. Infect Dis Obstet Gynecol. 2013;2013:184302.

Bertolino JG, Rangel JE, Blake RL Jr, Silverstein D, Ingram E. Inflammation on the cervical Papanicolaou smear: the predictive value for infection in asymptomatic women. Fam Med. 1992 Aug;24(6):447-52.

Day SJ, Deszo EL, Freund GG. Dual sampling of the endocervix and its impact on AutoCyte Prep endocervical adequacy. Am J Clin Pathol. 2002 Jul;118(1):41-6.
De Strooper LMA, Verhoef VMJ, Berkhof J, et al. Validation of the FAM19A4/mir124-2 DNA methylation test for both lavage- and brush-based self-samples to detect cervical (pre) cancer in HPV-positive women. Gynecol Oncol. 2016;141(2):341-347.
Ethos Biosciences. How to Set-Up and Conduct a Papanicolaou Stain for Both GYN and Non-GYN Samples. 2022. E disponueshme nw: https://www.ethosbiosciences.com/how-to-set-up-and-conduct-a-papanicolaou-stain.
Gavranović L, Novak SR, Bolanca IK. Causes and frequency of unsatisfactory cervicovaginal smears. Acta Med Croatica. 2011 Sep;65 Suppl 1:115-9.
Gustafsson L, Pontén J, Zack M, Adami HO. International incidence rates of invasive cervical cancer after introduction of cytological screening. Cancer Causes Control. 1997;8(5):755-763.
Islam S, West AM, Saboorian MH, Ashfaq R. Reprocessing unsatisfactory ThinPrep Papanicolaou test specimens increases sample adequacy and detection of significant cervicovaginal lesions. Cancer. 2004 Apr 25;102(2):67-73.
Lieu D. The Papanicolaou smear: its value and limitations. J Fam Pract, 1996; 42:391-399.
Lönnberg S, Hansen BT, Haldorsen T, Campbell S, Schee K, Nygård M. Cervical cancer prevented by screening: Long-term incidence trends by morphology in Norway. Int J Cancer. 2015;137(7):1758-1764.
Pangarkar MA. The Bethesda System for reporting cervical cytology. Cytojournal. 2022;19:28.
Parkin DM, Läärä E, Muir CS. Estimates of the worldwide frequency of sixteen major cancers in 1980. Int J Cancer. 1988;41(2):184-197.
Quiroga-Garza G, Satrum LS, Trujillo CJ, Mody DR, Ge Y. Common causes for unsatisfactory Pap tests in a high-risk population: insights into a yet unresolved problem in gynecologic cytology. J Am Soc Cytopathol. 2014 Sep-Oct;3(5):256-260.
Sachan PL, Singh M, Patel ML, Sachan R. A Study on Cervical Cancer Screening Using Pap Smear Test and Clinical Correlation. Asia Pac J Oncol Nurs. 2018 Jul-Sep;5(3):337-341.
Sahay K, Mehendiratta M, Rehani S, Kumra M, Sharma R, Kardam P. Cytological artifacts masquerading interpretation. J Cytol. 2013;30(4):241-246.
Solomon D, Davey D, Kurman R, Moriarty A, O'Connor D, Prey M, Raab S, Sherman M, Wilbur D, Wright T Jr, Young N; Forum Group Members; Bethesda 2001 Workshop. The 2001 Bethesda System: terminology for reporting results of cervical cytology. JAMA. 2002 Apr 24;287(16):2114-9.
Treacy A, Reynolds J, Kay EW, Leader M, Grace A. Has the ThinPrep method of cervical screening maintained its improvement over conventional smears in terms of specimen adequacy? Diagn Cytopathol. 2009 Apr;37(4):239-40.
Xhani A, Filipi K. Evaluation of cervical cytological abnormalities in Albanian population. Albanian Medical Journal, 2013; 4.