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ORIGINAL ARTICLE

Prevalence of cervical cytology abnormalities and its correlation with human papillomavirus infection: a single-institution five-year experience

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ABSTRACT

Aim To investigate the prevalence of cervical cytology abnormalities over a five-year period and examine its correlation with patients' age and presence/absence of HPV infection.

Methods Pap findings of 614 women with documented cervical abnormalities and Pap findings of HPV-positive women (regardless of whether the Pap test showed an intraepithelial abnormality) were included in this study.

Results The majority of women included in the study were aged 30-39 years, 224 (36.5%). The most common abnormalities included atypical squamous cells-ASC, 291(47.4%) and low-grade squamous intraepithelial lesions (LSIL), 93 (15.1%). Out of 614 women, 270 (43.9%) were positive for HPV. HR HPV positivity was found in 256 (41.7%). The proportion of HPV infection positivity decreases with age, especially in two oldest age groups (>60) (p=0.03). A higher ratio of Pap smear abnormalities in the group of patients >40 years of age was found (p=0.02). The youngest age group (<20 years) showed no other lesions than ASC, while women <40 had no squamous cell carcinoma (SCC) on Pap smear (p<0.01). Out of 256 HR HPV- positive women, 108 (42.2%) showed Pap abnormalities. HR HPV-positive women more commonly had high-grade squamous intraepithelial lesions (HSIL), but without statistical significance (p>0.05).

Conclusion The results obtained contribute to the knowledge about HPV prevalence and the prevalence of squamous cell abnormalities and confirm the necessity for further implementation of HPV vaccination and organized cervical cancer screening program at the national level.

Keywords: cervical dysplasia, Pap smear, screening

INTRODUCTION

According to available data, there are approximately 58,219 new cases of cervical cancer and 26,950 related deaths annually in Europe (1). Cervical cancer is the 6th most frequent cancer among women in Bosnia and Herzegovina (B&H) and the 2nd most frequent cancer among women between 15 and 44 years of age (2). Its prevention is possible by early detection and treatment of precancerous cervical lesions. The most common cervical cancer screening test globally is still the Papanicolaou (Pap) test together with colposcopy (3) and Human papillomavirus (HPV) testing, particularly in wealthy nations (4). Globally, the most widely used method for cervical cancer screening remains the Papanicolaou (Pap) test, commonly conducted in conjunction with colposcopy and Human papilloma virus (HPV) testing, particularly in high-income countries (4).

Although some advances in HPV vaccination and screening programs in B&H have been noticed, especially at the cantonal level, there is still a lack of complete national cervical cancer screening programs (5). In Southern Europe, about 3.8% of women in the general population are estimated to harbour cervical HPV-16/18 infection at a given time, which is considered to be responsible for 68.0% of invasive cervical cancers; however, data are not yet available on the HPV burden in the general population of our country (2). Implementation of the HPV Immunization Program in the Federation of Bosnia and Herzegovina began first in Canton Sarajevo in late 2022, with the aim of introducing mandatory vaccination (6).

The lack of complete data is also affected by the fact that a substantial portion of Pap smears is evaluated at private gynaecological clinics. The distribution of patients between private and public gynaecology clinics is unpredictable, along with the absence of a centralized database (5).

The aim of this study was to investigate the prevalence of cervical cytology abnormalities at our institution over a five-year period and to correlate it with patients' age and presence/absence of HPV infection, for patients who were tested.

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PATIENTS AND METHODS

Patients and study design

For this retrospective study, the database of the Department of Pathology, School of Medicine of the University of Sarajevo was searched for five years (2020-2024), and data were extracted on the total number of cervical Pap smears per year. In the period 2020-2024, a total number of 17.367 cervical smears were examined at our institution. The samples were obtained exclusively from patients attending 10 private gynaecological clinics, all situated within the Federation of B&H.

All women with documented Pap smear positive for cervical abnormalities and HPV-positive women (low-risk and/or high-risk HPV), regardless of whether the Pap test showed an intraepithelial abnormality, were included in this study. The age at the time of diagnosis was calculated for each patient according to medical records. The mean age, minimum, and maximum values were calculated. For further analytical purposes, the patients were categorized into age groups: younger than 20, 20-29, 30-39, 40-49, 50-59, 60-69, 70-79, 80-89. Where statistically appropriate, a dichotomous classification (<40 vs. ≥40 years) was also applied.

The data about HPV positivity (low or high risk) were extracted from the patients' referral. Some patients already had recorded data on HPV testing, while some were offered HPV testing based on cytological findings, so we obtained data on HPV positivity at repeated examinations. In the case of repeated Pap tests of the same patient, which showed deviations from the first finding, the finding with the most severe type of cervical epithelial lesion was included in the study.

Methods

Cervical smears were stained with Pap stain following the standardized procedure. The pap tests were interpreted by experienced pathologists and reported according to the Bethesda System classification of 2014 (7), and divided into the following categories: negative for intraepithelial lesion or malignancy (NILM), atypical squamous cells (ASC) including atypical squamous cells of undetermined significance (ASCUS) and ASC cannot exclude high-grade intraepithelial lesion (ASC-H), low-grade intraepithelial lesion (LSIL/CIN I), high-grade squamous intraepithelial lesion (HSIL) including moderate and severe dysplasia-cervical intraepithelial neoplasia /CIN2, CIN3/ and carcinoma in situ (CIS), squamous cell carcinoma (SCC), atypical glandular cells of undetermined significance (AGUS), and adenocarcinoma (ADC).

Samples of the cervical smear for HPV testing were taken during routine gynaecological examinations and tested for low-risk HPV (6, 11, 42, 43 and 44) and/or high-risk HPV types (16, 18, 31, 33, 34, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, and 70) (8).

Statistical analysis

Data were analysed according to type using the $\chi 2$ test and Spearman's correlation test, where appropriate. Statistical significance was observed for $p<0.05$.

RESULTS

PAP findings of 614 women with documented Pap smear results positive for cervical abnormalities and Pap findings of HPV-positive women (regardless of whether the Pap test showed an intraepithelial abnormality), were included in the study.

The mean age of the patients included in the study was 38.34±12.11 years (median age of 36 years), with the majority of patients under the age of 40, 384 (62.5%). The youngest patient was 19, and the oldest was 89. The largest proportion of women, 224 (36.5%), were aged 30-39. The smallest percentage comprised the group <20 and ≥80 years of age with three (0.5%) each, respectively.

Out of a total of 614 women included in the study, PAP findings of 437 (72.1%) women showed the presence of cervical epithelial abnormalities, while 177 (28.8%) women were negative for intraepithelial lesion or malignancy (NILM). When compared to the total number of Pap smears screened for cervical abnormalities, it presented 2.56% of cervical epithelial abnormalities in five years.

When analysed by a number of presented Pap abnormalities per year, a statistically significant decrease in the presence of cervical abnormalities in the observed period was noted (from 2020-2024) ($p<0.01$) (Table 1).

Table 1. Frequency of cervical abnormalities on Pap smear over a 5-year period

PAP finding	No (%) of women in the period					Total
	2020	2021	2022	2023	2024	
NILM	29 (18.4)	20 (17.9)	53 (44.5)	15 (23.1)	60 (37.5)	177 (28.8)
Positive for IEA	129 (81.6)	92 (82.1)	66 (55.5)	50 (76.9)	100 (62.5)	437 (71.2)
Total	158 (100.0)	112 (100.0)	119 (100.0)	65 (100.0)	160 (100.0)	614 (100.0)

NILM, Negative for Intraepithelial Lesion or Malignancy; IEA, intraepithelial abnormalities

The most common abnormalities included ASC (47.4%), followed by low-grade intraepithelial lesions (LSIL) with 15.1% of cases. HSIL lesions (including CIN II and CIN III) encompassed 6.8% of cases. Of the HSIL lesions, CIN III was defined in 3.4%, CIN II in 2.3% of patients, and 1.1% of patients had the HSIL label without further defining the subtype. For further easier data manipulation, we have grouped all these three categories as HSIL (6.8%). The least common lesions were SCC with 1.0% and AGUS with 0.8% of cases.

A total of 285 (out of 614; 46.4%), data about HPV testing were available. Out of 614 women, 270 (43.9%) were positive for HPV. HR HPV positivity was found in 256 (41.7%) while LR HPV positivity without HR HPV positivity was found in 14 (2.3%) patients. HR HPV-positive women were mostly younger than 40 years of age, 181 (70.7%).

The youngest HPV-positive woman was 19, and the oldest was 71. HPV-positive women were statistically significantly younger (mean age 36.29±10.15 years) compared to HPV-negative women (mean age 42.67±15.23) ($p=0.02$). The analysis showed that the proportion of HPV infection positivity decreases with age, particularly in patients aged 60 years or older (≥60). Thus, in the age groups <20 years and 50-59 years, all women were HPV positive (100%), while in the age group of 60-69 years, this percentage was 81.8%, and 50% in the age group of 70-79 years ($p=0.03$) (Figure 1).

The analysis of the presence/absence of intraepithelial abnormalities showed a higher ratio of Pap smear abnormalities in the group of patients older than ≥40 years of age ($p=0.02$) (Figure 2). Although ASC represented the most common type of Pap ab-

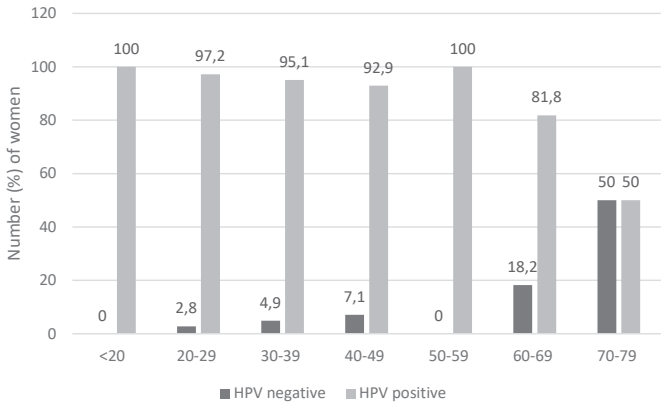

Figure 1. HPV infection distribution among age groups

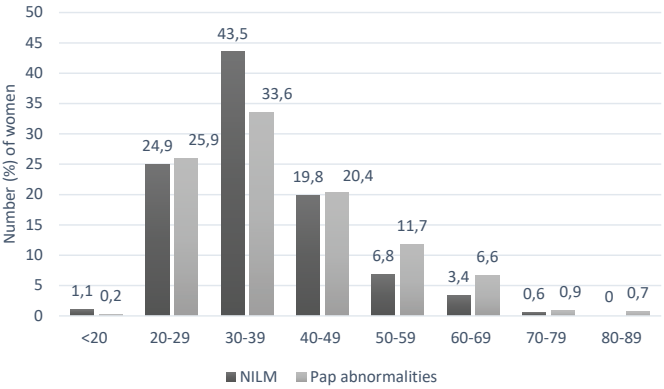

Figure 2. Prevalence of Pap intraepithelial abnormalities among age groups

normality found in all age groups, there is a significant difference in the prevalence of certain types of cervical epithelial lesions among age groups (p<0.01). The youngest age group (<20 years of age) showed no other lesions than ASC, while women aged <40 had no SCC on Pap smear. LSIL together with HSIL was predominant in age groups less than<40. HSIL and AGUS were absent in women ≥70 (Table 2).

Table 2. Distribution of intraepithelial abnormalities on Pap test by patients' age group

Age group (years)	Pap abnormalities					Total
	AGUS	ASC	LSIL	HSIL	SCC	
<20	0	1 (100.0)	0 (0.0)	0	0	1 (0.2)
20-29	1 (0.9)	71 (62.8)	30 (26.5)	11 (9.7)	0	113 (25.9)
30-39	1 (0.7)	94 (63.9)	35 (23.8)	17 (11.6)	0	147 (33.6)
40-49	1 (1.1)	59 (66.3)	16 (18.0)	11 (12.4)	2 (2.2)	89 (20.4)
50-59	2 (3.9)	40 (78.4)	8(15.7)	1 (2.0)	0	51 (11.7)
60-69	0	21 (72.4)	3 (10.3)	2 (6.9)	3 (10.3)	29 (6.6)
70-79	0	3 (75.0)	1 (25.0)	0	0	4 (0.9)
80-89	0	2 (66.7)	0	0	1 (33.3)	3 (0.7)
Total	5 (1.1)	291 (66.6)	93 (21.3)	42 (9.6)	6 (1.4)	437 (100.0)

AGUS, atypical glandular cells of undetermined significance; ASC, atypical squamous cells; LSIL low-grade squamous intraepithelial lesion; HSIL, high-grade squamous intraepithelial lesion; SCC, squamous cell carcinoma

Comparison according to age groups showed that AGUS, ASC and SCC were more prevalent in the age group ≥40 years, while LSIL and HSIL were more prevalent in the age group <40 years (p=0.008) (Figure 3).

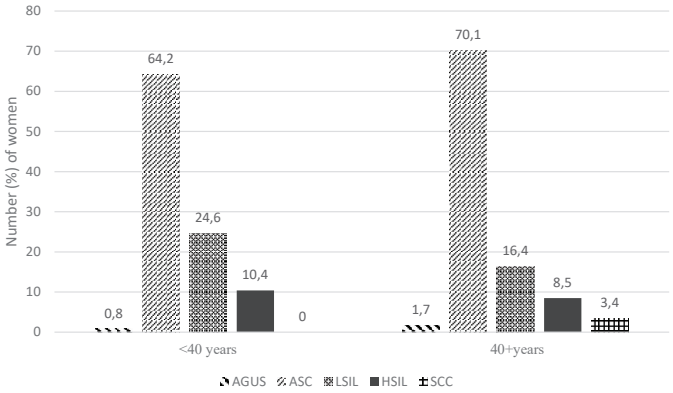

Figure 3. Frequency of the type of intraepithelial lesion in the group of women younger and older than 40 years of age

Out of 256 HR HPV- positive women 108 (42.2%) showed Pap abnormalities. Statistical analysis showed no significant association between HR HPV positivity and type of cervical lesion, although it was noticed that HR HPV-positive women more commonly had HSIL lesions, compared to those who were HR HPV-negative (p>0.05).

DISCUSSION

Crude incidence rates of cervical cancer per 100,000 women per year in Bosnia and Herzegovina are 18.6, which is much higher compared to other countries in the region, such as Slovenia, Albania and Croatia with incidence rates of 9.96, 9.41 and 15.8, respectively (2). The annual number of cervical cancer cases in Bosnia and Herzegovina is 312, and the annual cervical cancer deaths number is 153 (2). An average age-standardized incidence rates per 100,000 females of 12.48 in the Federation of B&H, and 22.44 in Republika Srpska was reported for the period January 2016 to December 2020 (9).

The reduction in the number of cervical abnormalities observed over the five-year period may be attributed to the fact that the analysed samples were from women who regularly attended gynaecological check-ups in private clinics, suggesting that consistent preventive care contributed to the decreased prevalence.

Similar to other studies, we found atypical squamous cells (ASC) followed by low-grade intraepithelial lesions (LSIL) as the most common Pap smear abnormalities (4,5,10,11).

Because women aged up to 40 are of reproductive age and are sexually active, it is expected to have more gynaecological examinations and thus more Pap smear abnormalities (5). Our data confirm this hypothesis, since in our study patients younger than 40 were the most prevalent age group (62.5%), and the most common and important preinvasive lesions (LSIL and HSIL) were the most prevalent in this age group.

Although ASC represented the most common type of Pap abnormality found in all age groups, there is a significant difference in the prevalence of certain types of cervical lesions among the age groups. Women aged less than 20 showed no other lesions than atypical squamous cells, while women aged less than 40 had no invasive lesions- SCC on Pap smear. These findings can be explained by the fact that the development of cervical carcinoma from preinvasive lesions requires years, thus being preventable disease by Pap smear regular testing (11).

In Southern Europe the prevalence of HRHPV (HPV 16/18) positivity among LSIL is 25.4 (95% CI=24.6-26.2), while among HSIL it is 53.2 (2). Data show that HPV infections are most prevalent in young, sexually active women and that their

frequency tends to decline significantly beyond the age of 45 (12,13), especially since some women eradicate infection by competent immune system very early, leading to decrease of HPV prevalence even after 25 years of age (12).

One of the reasons why the prevalence of HPV in B&H is still unclear is because the country lacks a standardised database of Pap test or HPV test results, especially to consolidate the data obtained from private and public health institutions (5). Our study also showed that the proportion of HPV infection positivity decreases with age, especially in the two oldest age groups (women older than 60). The finding that HPV-positive women were significantly younger than HPV-negative women is in line with previous studies examining the prevalence of HPV (14,15).

Since ASC was the most common type of cervical abnormality found in our study, many of the patients with ASC were tested for HPV. Possible ways of ASC management can include follow-up with cytology, HPV testing or colposcopy (16). In case of multiple Pap smears of the same patient over a 5-year period, we correlated the most difficult intraepithelial abnormality with HPV test results.

Out of 614 women, 270 (43.9%) were positive for HPV. HR HPV positivity was found in 256 (41.7%) women. A slightly lower prevalence of HR HPV positivity (16 and 18) was recorded in the study of Sadiković et al. (5), the reason for which may be the inclusion of women younger than 40 in the study. In Serbia, an overall HPV prevalence of 41.3% was found among women with normal Pap and speculum findings without a history of precancerous lesions (17). Similar results for ten-year period in overall HPV positivity showed other studies, also from Serbia (for the Vojvodina region), with HPV positivity found in 43.3% of patients (18). Considering that the most prevalent age group in our study was 30-39 age, we found a similar percentage of HR HPV positivity in patients younger than 40 (70.7%) as previous studies from Bosnia (5) and the neighbouring countries (17). Out of 256 HR HPV-positive women 42.2% showed Pap abnormalities. Our findings, although without reaching statistical significance, showed that HR HPV is most prevalent in patients with HSIL cytological findings, similar to other studies (5,17). Unlike LSIL lesions which tend to resolve within a few years, HSILs are linked to persistent infection and an increased risk of progression to invasive cervical cancer, particularly if the persistent infection is a high-risk genotype like HPV 16 or 18 (19).

Introducing screening programs at the national level in our country would probably lead to an increase of abnormal cytology findings on Pap smears together with the increase in HPV positivity, but would decrease the incidence and mortality, as it is the case with developed countries (20).

In conclusion, this study contributes to knowledge about the prevalence of HPV infection and squamous cell abnormalities among women regularly checked in different private institutions in Canton Sarajevo especially since we lack data flow between private and public health institutions. However, we are also aware that this could be a potential limiting factor since it does not represent the true state of the nation's condition and might be influenced by the level of awareness about the disease and the need for regular gynaecological examinations in different socioeconomic groups. Insight into HPV prevalence is important to ensure access to a sufficient amount of vaccines against HPV and to achieve cooperation with the public and private sectors, in order to increase vaccine coverage and develop national guidelines for the treatment of this type of cancer, as well as systematic monitoring of results to ensure progress.

AUTHOR CONTRIBUTIONS

Conceptualization, N.Č.; Data curation, N.Č., A.B. and E.M.; Formal analysis, N.Č. and A.B.; Investigation, M.D. and M.B.; Methodology, N.Č., E.L.S., S.K.V., and E.M.; Writing – original draft, N.Č. and E.M.; Writing – review & editing, S.K.V., E.L.S; Supervision, S.K.V., M.D, M.B. All authors have read and agreed to the published version of the manuscript.

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TRANSPARENCY DECLARATION

Conflict of interests: None to declare.

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