Age-specific prevalence of Human Papillomavirus type 16/18 infections, abnormal cytology and cervical intraepithelial neoplasia among screened women in Tbilisi

Kiguradze E.[1,3], Skhirtladze T.[3], Chkhartishvili N.[2], Gogoladze T.[3], Chikhladze N.[1], Alibegashvili T.[3]

Abstract

Objective: The objective of this study was to estimate the age-specific prevalence of Human Papillomavirus (HPV) type 16/18 infections, abnormal Pap results, and cervical intraepithelial neoplasia CIN2+ in women screened in Tbilisi, Georgia. Additionally, the study aimed to evaluate the association between HPV genotyping, Pap test results, and the presence of CIN2+ disease, providing valuable information for clinical decision-making and treatment strategies.

Methods: This cross-sectional study recruited women at the locations of National Screening Centre in Tbilisi. Participants underwent HPV testing using polymerase chain reaction (PCR) to detect HPV types 16 and 18. PAP tests were conducted to evaluate cervical abnormalities. Colposcopy was performed in women with atypical PAP results and/or HPV-positive tests and in case of abnormal colposcopy findings biopsy samples were collected for histological analysis to determine the presence of CIN2+ disease. Statistical analyses were performed to estimate prevalence of HPV infection, abnormal PAP results, and CIN2+ disease. Multivariate analysis was conducted to assess associations between HPV types 16/18 and abnormal PAP result with CIN2+ disease.

Results: Among 998 participants enrolled, 1.3% had invalid HPV genotyping test results, and 0.4% had invalid PAP test results, leading to their exclusion from further analysis. Among the 981 women with complete data, the prevalence of high-risk HPV (Hr-HPV) was 11.3%, with HPV genotypes 16/18 accounting for 3.4% (95% CI: 2.3%-4.7%). The prevalence of any abnormal PAP result was 11.0% (95% CI: 9.1%-13.1%), and the prevalence of CIN2+ diseases was 1.3% (95% CI: 0.7%-2.2%). Stratification by age categories showed a higher prevalence of Hr-HPV and abnormal PAP results among women aged 30-39, which decreased in older age groups. Statistically significant differences were observed for HPV genotypes 16/18 and abnormal Pap results, but not for ≥CIN2 disease. The Poisson regression model indicated a strong association between HPV genotypes 16/18 and CIN2+ disease (PR 49.90, 95% CI: 18.45-134.92, $p<0.0001$). Abnormal PAP test results showed a significant association in univariate analysis but not in the multivariate model. No association was found between age and CIN2+ disease.

Conclusion: The prevalence of HPV 16/18 types and abnormal PAP results varied with age, with higher rates observed in women aged 30-39, which decreased in older age groups. No significant differences were found in the prevalence of CIN2+ across age groups. HPV genotyping may serve as a more reliable predictor of ≥CIN2 disease compared to PAP testing, highlighting the importance of implementing HPV screening in Georgia. **(TCM-GMJ December 2023; 8 (2):P47-P51)**

Keywords: HPV; PAP; CIN2+ disease; Cervical Cancer; Georgia.

Introduction

Cervical cancer (CC) is a significant public health concern, ranking as the fourth most common cancer among women worldwide (1). CC develops as a result of long-term persistence of sexually transmitted high-risk human papillomavirus (Hr-HPV) infection, which can progress through precancerous stages such as cervical intraepithelial neoplasia grade 2 and worse (CIN2+). About 90% of new CC cases and deaths in 2020 occurred in low- and middle-income countries, highlighting the urgent need for effective prevention and control strategies. In Georgia, CC is the fifth most common cancer among women (2).

It is well-established that the distribution of HPV genotypes in cervical cancers varies by country and region (3,4). HPV16 and HPV18 are the most prevalent genotypes worldwide (5,6,7) and are responsible for development of 70% of CC cases (8). Information on the population prevalence of Hr-HPV and CIN2+ is crucial for understanding the burden of disease and guiding appropriate screening and prevention strategies. It also provides key inputs for resource allocation and quality assurance of screening programs. Population-based data on cervical precancers are also important for assessing the impact of HPV vaccination in the future.

The first attempt to study HPV prevalence in Georgia was conducted by the International Agency for Research on Cancer (IARC) in 2007, which evaluated the HPV types among 1344 women and revealed that the prevalence of Hr-HPV was 8.6%, with HPV16/18 accounting for 1.9% of cases (9). However, since that

From the [1]Faculty of Medicine, Ivane Javakhishvili Tbilisi State Universityaris; Tbilisi, [2]Georgia; Infectious Diseases, AIDS and Clinical Immunology Research Center, Tbilisi, Georgia; [3]National Screening Center, Tbilisi, Georgia;
Received October 3, 2023; accepted December 10 , 2023.
Address requests to: Eter Kiguradze
E-mail: eter.kiguradze522@med.tsu.edu.ge

study, there have been no updates on HPV prevalence in Georgia, despite the importance of such data for effective prevention and control of cervical cancer. Georgian Cervical Cancer Screening program, which was launched in 2008, is based on conventional cytology. In this article, we present the findings from a recent study of 998 women recruited from October 2021 to May 2022 for routine screening round in the Georgian National Screening Center in Tbilisi, aimed at estimating the prevalence of high-risk HPV, abnormal PAP test and cervical intraepithelial neoplasia grade 2 or worse (CIN2+). Our study provides an updated estimate of HPV prevalence and contributes to the understanding of the burden of cervical precancers in Tbilisi.

Aim

To estimate prevalence (including age-specific prevalence) of Human Papillomavirus type 16/18 infections, abnormal PAP results and cervical intraepithelial neoplasia CIN2+ among screened women in Tbilisi.

Materials and Methods

Study design and procedures

This was a cross-sectional, prospective study conducted at three sites of National Screening Centre in different geographic locations in Tbilisi from October 2021 to May 2022. Women between 30 and 60 years of age, who were residents of Georgia, scheduled for a screening round, and willing to participate, were eligible for inclusion in the study. Women with a history of cervical cancer, who were pregnant, lacked a cervix, were being followed up for a cervical lesion, or were unable to provide informed consent were excluded from the study. A total of 1000 eligible women were selected from every second woman who attended all three facilities of the Georgian National Screening Center (GNSCs) for a routine screening round.

During one visit, all enrolled women underwent conventional PAP smear from the cervix with Ayre wood spatula and endocervical brush, as well as additional scrape with a new brush for HPV testing using the specially designed trident-shaped Cervex Brush. The samples were taken by a gynecologist. The cytology screening was performed according to Georgian National Guidelines, and the conventional PAP smear glasses were referred to GNSC Cytolab, with results reported according to the Bethesda 2014 system (10).

HPV Testing and Colposcopy: HPV testing was done using the Cobas 4800 system, which is an automated qualitative *in-vitro* test for the detection of HPV DNA in patient specimens. The test utilized amplification of target DNA by the Polymerase Chain Reaction (PCR) and nucleic acid hybridization for the detection of 14 high-risk HPV types in a single analysis. Women with negative results on both Pap and HPV tests were referred for a further round of screening after three years, while those with any positive screening results or suspicion of cervical cancer during visual examination of the cervix were referred to colposcopy. During colposcopy examination, a standard 5% acetic acid test and Shiller's test were performed. The results of colposcopy examination were reported using IFCPC 2011 nomenclature (11).

Biopsy and Histology: Cervical punch biopsy was taken only in the case of abnormal colposcopy, while endocervical curettage was performed in cases of AGC at PAP result. Biopsy tissue was stained according to the standard protocol with Hematoxylin&Eosin and was read by two pathologists without knowledge of each other's diagnoses. Histology diagnoses were categorized following the CIN classification system. If the biopsy revealed CIN1 lesions or less, the women were referred for follow-up visit in 12 months. The final endpoint of the study was the histological diagnosis of CIN2 or worse, and those women were advised treatment - Large Loop Excision of Transformation Zone (LLETZ).

Statistical Analysis

Outcomes of interest were prevalence of HPV genotype 16 and/or 18, abnormal Pap results and cervical intraepithelial neoplasia CIN2+. Prevalence with 95% Confidence Intervals were calculated using exact binomial approach. Bivariate comparisons were tested using Pearson's chi-square or Fisher's exact tests as appropriate. Poisson regression model with robust variance estimates was used to assess association between HPV and Pap testing with the presence of ≥CIN2 disease. All statistical analyses were performed using SAS 9.4. P value <0.05 was considered statistically significant.

Ethics Statement

The study was approved by the Institutional Review Board of the Infectious Diseases, AIDS, and Clinical Immunology Research Center (OHRP #: IRB00006106). All participants provided written informed consent.

Results

Study approached 1,000 women, but due to the damage of Roche Cell Collection Medium vials, two participants were excluded, thus 998 women remained in the study (median age 43, IQR: 37-49, years). Among them, 13 (1.3%) women had invalid HPV genotyping test results, 4 (0.4%) women had invalid PAP test result and consequently were excluded from further analysis (Figure 1). Among 981 women with complete data the prevalence of Hr-HPV test was 11.3% and among them HPV genotype 16/18 was 3.4% (95% CI: 2.3%-4.7%), prevalence of any abnormal PAP result (ASCUS, ASC-H, LSIL, HSIL, AGC) - 11.0% (95% CI: 9.1%-13.1%) and of ≥CIN2 diseases- 1.3% (95% CI: 0.7%-2.2%) (Figure 1).

Stratification by age categories showed age dependent pattern with higher prevalence observed among women aged 30-39 decreasing in age groups of 40-49 and 50+ years. Statistically significant differences were noted for HPV genotype 16/18 ($p=0.01$) and abnormal PAP test

result (p=0.003), but not for CIN2+ disease (p=0.82) (Figure 2).

Poisson regression model was used to identify association of HPV genotyping and PAP testing results with the presence of CIN2+disease. Analysis showed that HPV genotype 16/18 was strong predictor of CIN2+ disease with prevalence ratio (PR) 49.90 (95% CI: 18.45-134.92, p<0.0001). Abnormal PAP test result showed significant association in univariate analysis, (PR 3.59, 95% CI: 1.13-11.47, p=0.03), but not in multivariate model (PR 3.55, 95% CI: 0.94-13.43, p=0.06). No association was found between age and CIN2+ disease in either univariate or multivariate models (Table 1).

Discussion

Our study shows higher prevalence of HPV and abnormal PAP results in women younger than 40 years old, with clear association between the presence of HPV 16/18 and CIN2+ disease.

The results of a meta-analysis suggest that the highest prevalence of HPV occurs at the age of 25 years, possibly due to changes in sexual behavior (12). In some regions, a bimodal distribution of cervical cancer has been observed, where an initial outbreak of HPV is seen after sexual initiation, followed by a plateau in adult age, and then a second peak after the age of 45 (3). Persistent infection with high-risk HPV types over time can lead to the development of CIN. The major mechanisms by which HPV contributes to carcinogenesis involve the viral oncoproteins E6 and E7, which interfere with major tumor suppressor genes and are associated with changes in host and viral DNA methylation. These interactions are associated with changes in key cellular pathways that regulate genetic integrity, cell adhesion, immune response, apoptosis, and cellular control (13). Additionally, the age-standardized prevalence of HPV has been shown to be significantly lower in women over the age of 30 compared to those under 30 in other studies (14).

The prevalence of HPV16/18 in our study – 3.4%, is in line with studies from Brazil and England where was detected prevalence of HPV16/18 correspondingly 3.26% and 4% (15,16), is a little bit higher than it was reported in Australia - 2% (17), but lower than figures detected in Canada – 6.2% (18). At the same time figures of prevalence of HPV16/18 almost twice higher than findings of previous study conducted in Tbilisi in 2007 – 1.8% (9)

Our study, as well as international studies (15,17), demonstrate that the prevalence of HPV 16/18 decreases with age. Specifically, in women aged 30-39 years, it is almost 2 times higher than in women aged 40-49 years (5.6% vs 3%, p=0.01), and 5.6 times higher than in women aged 50 years and older (5.6% vs 1%, p=0.01). The prevalence of PAP atypia also follows a similar trend in different age categories, with the highest occurrence in women aged 30-39 years (14.8%), decreasing in women aged 40-49 years (11.1%), and being almost 2.5 times lower in women aged 50 years (5.4%) (p=0.003). But our study revealed a different rate of abnormal Pap test prevalence (11%) compared to studies conducted in other European and Asian countries, where its rate was lower and varies from 1.8% to 7.3% (in Turkey 1.8%, in the United Emirates - 4.9%, in Romania 5.9%, in Iran 4.04%, in Italy 2.4%, in Belgium 3.7% , in Croatia 7.3%. (19-25)

Prevalence of CIN2+ disease in our study was 1.3% and had no differences among age groups (p=0.82). This discrepancy in the age distribution of HPV prevalence and abnormal Pap results versus CIN2+ results can be explained with several facts:

Firstly, it is possible that the risk factors for acquiring HPV infection and developing CIN2+ lesions are different. HPV infection is primarily transmitted through sexual contact, and younger women may be more likely to engage in risky sexual behavior. In contrast, the development of CIN2+ lesions may depend on other factors, such as immune status or genetic predisposition, which may not be related to age. Secondly, the natural history of HPV infection may differ from the development of CIN2+ lesions. While most HPV infections clear up on their own within a year or two, some persistent infections can lead to the development of precancerous lesions. The time between HPV infection and the development of CIN2+ lesions can vary widely, and may not be strongly related to age. It's also possible that other unmeasured factors, such as smoking or other co-infections, may be confounding the association between age and CIN2+ risk. (26-30)

Multivariate analysis showed strong association between the presence of HPV /16/18 and CIN2+ diseases, with a prevalence ratio of 49.9 and a p value of <0.0001. This clearly shows predictive value of HPV genotyping in predicting CIN2+ disease. With regard to PAP testing, abnormal values showed association with CIN2+ disease in univariate analysis, but lost significance in multivariate model. Nevertheless, the level of association between abnormal PAP result and CIN2+ disease was borderline at p value of 0.06, which means that the value of PAP test cannot be fully ruled-out), suggesting that there may be a trend towards a significant association. It's worth noting that the results of the multivariate analysis may provide a more accurate and reliable estimate of the independent effect of each predictor variable on the outcome, as it takes into account the potential confounding effects of other factors. However, the interpretation of the results should be cautious and consider the specific research question, study design, and potential sources of bias and uncertainty. Overall, our results support use of HPV genotyping as primary screening method, but PAP test can be also valuable if genotyping is not available.

High p-value for the prevalence of age categories indicates that age of women was not the predictor of CIN2+ diseases and it can be found in all age groups from 30 to 60.

Conclusion

The prevalence of HPV 16/18 types and abnormal PAP results were found to be age-dependent, with higher rates observed among women aged 30-39 years, decreasing in older age groups. However, no significant differences were observed in the prevalence of CIN2+ across different age groups. These findings suggest the importance of regular screening for cervical cancer. The study also highlights the need for further research to understand the mechanisms behind the age-dependent patterns of HPV infection and cervical cancer. Also, study results suggest that HPV genotyping may be a more reliable predictor of CIN2+ disease than PAP testing results and proves how important is implementation of HPV screening in Georgia.

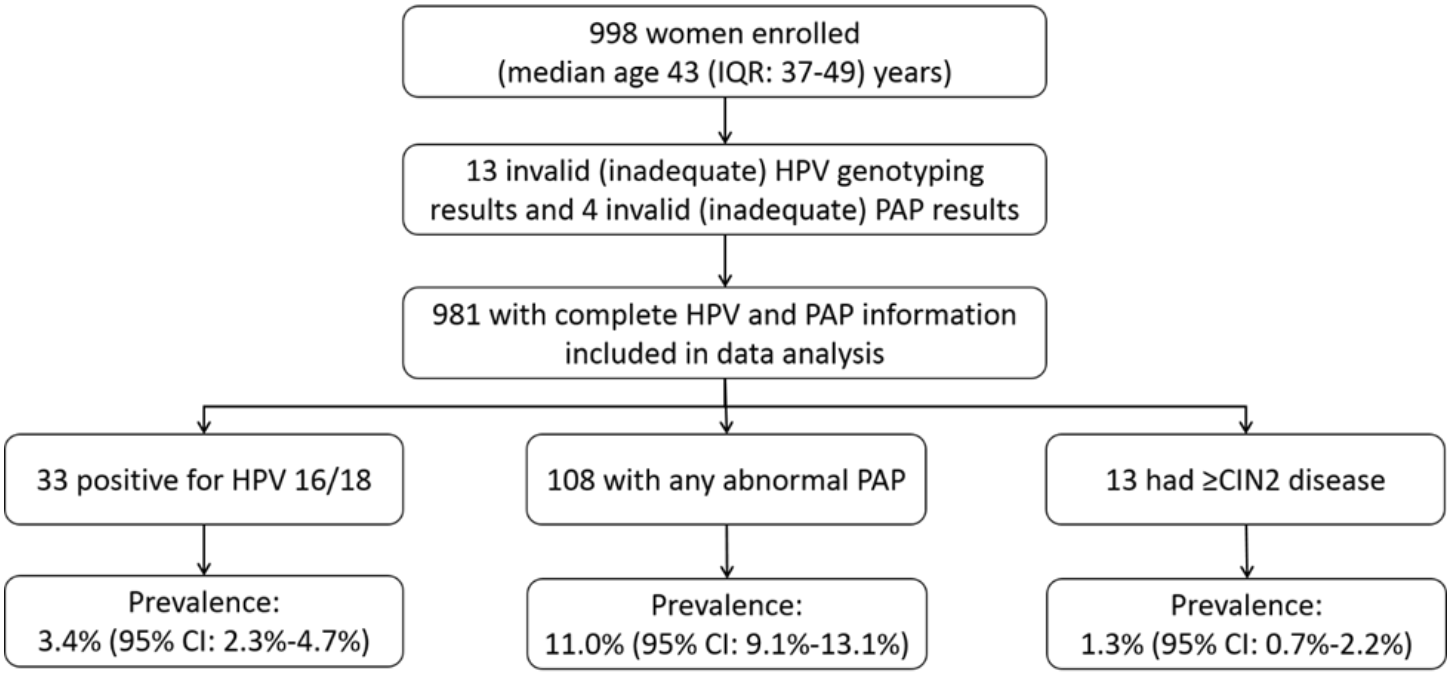

Figure 1. Study population flow

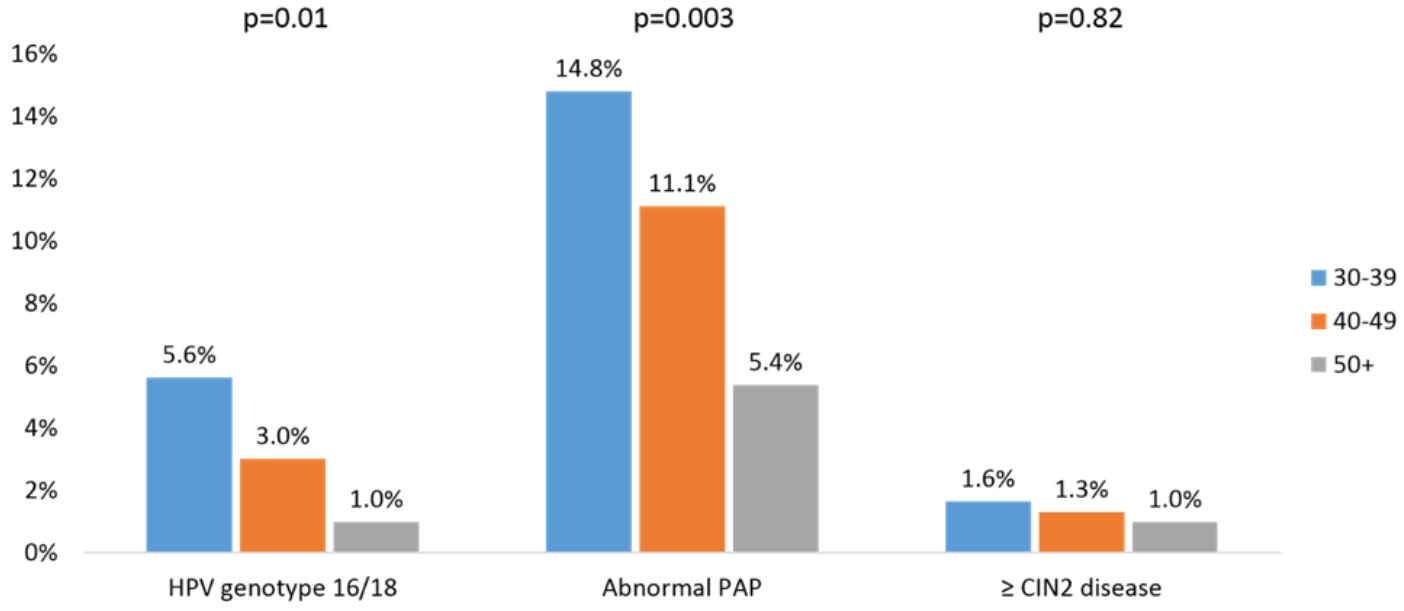

Figure 2. Prevalence of HPV genotype 16/18, abnormal PAP and CIN2+disease by age categories

Table 1. Factors associated with the presence of CIN2+disease

	Univariate model		Multivariate model	
	Prevalence ratio (95% CI)	**p value**	**Prevalence ratio (95% CI)**	**p value**
HPV				
Genotype 16/18	45.96 (15.89-132.94)	<0.0001	49 .90 (18.45-134.92)	<0.0001
No genotype 16/18	1		1	
PAP				
Abnormal PAP	3.59 (1.13-11.47)	0.03	3.55 (0.94-13.43)	0.06
Normal PAP	1		1	
Age categories				
30-39	1.72 (0.34-8.77)	0.52	0.56 (0.10-3.26)	0.51
40-49	1.34 (0.27-6.58)	0.72	0.65 (0.11-3.98)	0.65
50-59	1		1	

References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2021:71:209–49. doi:10.3322/caac.21660.
2. Malignant tumors in Georgia 2015-2021 https://www.ncdc.ge/#/pages/file/7bb73fc7-12bc-4b09-a18a-7b17b9cace94
3. Bruni L, Diaz M, Castellsagué X, Ferrer E, Bosch FX, de Sanjosé S. Cervical human papillomavirus prevalence in 5 continents: meta-analysis of 1 million women with normal cytological findings. J Infect Dis. 2010 Dec 15;202 (12):1789-99. doi: 10.1086/657321. Epub 2010 Nov 10. PMID: 21067372.
4. Poljak M, Seme K, Maver PJ, Kocjan BJ, Cuschieri KS, Rogovskaya SI, Arbyn M, Syrjänen S. Human papillomavirus prevalence and type-distribution, cervical cancer screening practices and current status of vaccination implementation in Central and Eastern Europe. Vaccine. 2013 Dec 31;31 Suppl 7:H59-70. doi: 10.1016/j.vaccine.2013.03.029. PMID: 24332298.
5. De Sanjosé S, Diaz M, Castellsagué X, Clifford G, Bruni L, Muñoz N, Bosch FX. Worldwide prevalence and genotype distribution of cervical human papillomavirus DNA in women with normal cytology: a meta-analysis. The Lancet infectious diseases. 2007 Jul 1;7(7):453-9.
6. Cohen PA, Jhingran A, Oaknin A, Denny L. Cervical cancer. The Lancet. 2019 Jan 12;393(10167):169-82.
7. Castellsagué X, Iftner T, Roura E, Vidart JA, Kjaer SK, Bosch FX, Muñoz N, Palacios S, Rodriguez MS, Serradell L, Torcel-Pagnon L. Prevalence and genotype distribution of human papillomavirus infection of the cervix in Spain: the CLEOPATRE study. Journal of medical virology. 2012 Jun;84 (6):947-56.
8. De Martel C, Plummer M, Vignat J, Franceschi S. Worldwide burden of cancer attributable to HPV by site, country and HPV type. International journal of cancer. 2017 Aug 15; 141(4):664-70.
9. Alibegashvili T, Clifford GM, Vaccarella S, Baidoshvili A, Gogiashvili L, Tsagareli Z, Kureli I, Snijders PJ, Heideman DA, van Kemenade FJ, Meijer CJ. Human papillomavirus infection in women with and without cervical cancer in Tbilisi, Georgia. Cancer epidemiology. 2011 Oct 1; 35(5):465-70.
10. Nayar R, Wilbur DC. The pap test and Bethesda 2014. Acta cytologica. 2015; 59(2):121-32.
11. Bornstein J, Bentley J, Bösze P, Girardi F, Haefner H, Menton M, Perrotta M, Prendiville W, Russell P, Sideri M, Strander B. 2011 colposcopic terminology of the International Federation for Cervical Pathology and Colposcopy. Obstetrics & Gynecology. 2012 Jul 1; 120(1):166-72
12. Crosbie EJ, Einstein MH, Franceschi S, et al Human papillomavirus and cervical cancer. Lancet. 2013; 382:889–99. doi: 10.1016/S0140-6736(13) 60022-7.
13. Mittal S, Banks L Molecular mechanisms underlying human papillomavirus E6 and E7 oncoprotein-induced cell transformation. Mutat Res Rev Mutat Res. 2017; 772:23–35. doi: 10.1016/j.mrrev.2016.08.001
14. Burchell AN, Winer RL, de Sanjosé S, Franco EL. Epidemiology and transmission dynamics of genital HPV infection. Vaccine. 2006 Aug 21; 24:S52-61
15. Rebolj M, Mathews CS, Pesola F, Cuschieri K, Denton K, Kitchener H, HPV Pilot Steering Group, Appleyard TL, Cruickshank M, Ellis K, Evans C. Age-specific outcomes from the first round of HPV screening in unvaccinated women: observational study from the English cervical screening pilot. BJOG: An International Journal of Obstetrics & Gynaecology. 2022 Jul; 129(8):1278-88.
16. Teixeira JC, Vale DB, Discacciati MG, Campos CS, Bragança JF, Zeferino LC. Cervical Cancer Screening with DNA-HPV Testing and Precancerous Lesions Detection: A Brazilian Population-based Demonstration Study. Revista Brasileira de Ginecologia e Obstetrícia. 2023 Apr 14; 45:21-30.
17. Brotherton JM, Hawkes D, Sultana F, Malloy MJ, Machalek DA, Smith MA, Garland SM, Saville M. Age-specific HPV prevalence among 116,052 women in Australia's renewed cervical screening program: a new tool for monitoring vaccine impact. Vaccine. 2019 Jan 14; 37(3):412-6
18. Jiang Y, Hanley B, Brassard P, Severini A, Lo J, O'Donovan S, Duncan L, Luo ZC, Tohme S, Mao Y, Morrison H. Human papillomavirus infection and the association with abnormal Pap findings in Yukon, Canada. Journal of Lower Genital Tract Disease. 2013 Jul 1; 17(3):346-53.
19. 1Stolnicu S, Musca S, Micu D, Micu L, Moldovan C, Puscasiu L. Prevalence of abnormal Pap smears in a consecutive and previously unscreened population in Romania. International Journal of Gynecology & Obstetrics. 2014 Feb 1; 124(2):156-9.
20. Maleki A, Ahmadnia E, Avazeh A, Mazloomzadeh S, Molaei B, Jalilvand A. Prevalence of abnormal papanicolaou test results and related factors among women living in Zanjan, Iran. Asian Pacific journal of cancer prevention. 2015;16(16):6935-9.
21. Turkish Cervical Cancer and Cervical Cytology Research Group. Prevalence of cervical cytological abnormalities in Turkey. Int J Gynecol Obstet 2009; 106(3):206–9.
22. Giorgi Rossi P, Ricciardi A, Cohet C, Palazzo F, Furnari G, Valle S, et al. Epidemiology and costs of cervical cancer screening and cervical dysplasia in Italy. BMC Public Health 2009; 9:71.
23. Skopljanac-Macina L, Mahovlić V, Ovanin-Rakić A, Barisić A, Rajhvajn S, Juric D, et al.Cervix cancer screening in Croatia within the European Cervical Cancer Prevention Week. Coll Antropol 2010; 34(2):613–7.
24. Arbyn M, Van Nieuwenhuyse A, Bogers J, De Jonge E, De Beeck LO, Matheï C, et al. Cytological screening for cervical cancer in the province of Limburg, Belgium. Eur J Cancer Prev 2011; 20(1):18–24.
25. Al Zaabi M, Al Muqbali S, Al Sayadi T, Al Ameeri S, Coetsee K, Balayah Z, Ortashi O. Age specific cytological abnormalities in women screened for cervical cancer in the Emirate of Abu Dhabi. Asian Pacific Journal of Cancer Prevention. 2015; 16(15):6375-9.
26. Singh K, Balega J. Cancer of the uterine cervix. In: Shaw RW, Luesley D, Monga A, editors. Gynaecology. 4th ed. Elsevier Limited 2011; 582-83
27. Louie KS, de Sanjose S, Diaz M, Castellsagué X, Herrero R, Meijer CJ, et al. Early age at first sexual intercourse and early pregnancy are risk factors for cervical cancer in developing countries. Br J Cancer. 2009;100:1191–7
28. Leo PJ, Madeleine MM, Wang S, Schwartz SM, Newell F, Pettersson-Kymmer U, Hemminki K, Hallmans G, Tiews S, Steinberg W, Rader JS. Defining the genetic susceptibility to cervical neoplasia—A genome-wide association study. PLoS genetics. 2017 Aug 14;13(8):e1006866.
29. Ramachandran D, Dörk T. Genomic risk factors for cervical cancer. Cancers. 2021 Oct 13;13(20):5137.
30. Akram Husain RS, Ramakrishnan V. A review of risk factors in the development of cervical malignancy. Cancer Sci Res Open Access. 2016; 3(1):1-4.