Comparative study of pap smear and polymerase chain reaction tests for human papillomavirus screening in Bahrain

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Abstract

Background: Cervical cancer remains a global health concern, particularly in low- and middle-income countries, including Bahrain, where human papillomavirus (HPV) infection is the leading cause.

Aim: To compare the diagnostic accuracy of cervical cytology (pap smear) and molecular HPV detection using polymerase chain reaction (PCR) in identifying cervical abnormalities among women in Bahrain.

Methods: We retrospectively analysed 320 pap smear samples from the Salmaniya Medical Complex laboratory. Cytological findings were compared with HPV PCR results, using PCR testing as the gold standard. Sensitivity, specificity, positive predictive value and negative predictive value were calculated. Statistical analyses included chi-square and Fisher's exact tests.

Results: Among the 320 samples, 56 (17.5%) were HPV-positive and 264 (82.5%) were HPV-negative. PCR showed higher sensitivity (95.5%) but had reduced moderate specificity (67.4%), with a positive predictive value for detecting histopathological lesions of 94.6% and a negative predictive value of 96.9%. Pap smears identified 57.5% as negative for intraepithelial lesion or malignancy and 42.5% with abnormalities. HPV-positive cases had higher abnormal cytology rates, and histopathology confirmed lesions in some HPV-positive cases despite normal cytology results.

Conclusion: Combining HPV PCR with pap smear enhances cervical cancer detection. Tailored screening programmes based on individual risk factors are recommended to reduce the disease burden.

Keywords: cervical cancer, HPV, pap smear, PCR, sensitivity, specificity, cytology, Bahrain

Citation: Saeed NK, Alshaikh S, Al-Beltagi M. Comparative study of pap smear and polymerase chain reaction tests for human papillomavirus screening in Bahrain. East Mediterr Health J. 2025;31(7):453-462. https://doi.org/10.26719/2025.31.7.453.

Received: 16/09/2024; Accepted: 22/01/2025.

Introduction

Cervical cancer is the fourth most common cancer among women worldwide. Despite advancements in screening and prevention, it remains a leading cause of morbidity and mortality, particularly in resource-limited settings *(1)*. Similar to global trends, cervical cancer ranks fourth among cancers affecting women in Bahrain, with an annual incidence rate of approximately 4.3 per 100 000 individuals and an age-standardized incidence rate of 5.9. Among the Gulf Corporation Council (GCC) countries, Bahrain has the second highest incidence rate after the United Arab Emirates *(2)*. Timely detection and interventions are critical to addressing this public health challenge.

Persistent infection with high-risk human papillomavirus (HPV) genotypes, notably HPV 16, 18 and 31, is the primary cause of cervical cancer, detected in over 90% of cases *(3)*. Screening, particularly pap smear testing, have been instrumental in reducing cervical cancer incidence *(4)*. Over the past 40 years, pap smear screening has led to a 50% reduction in cervical cancer cases. Screening should be initiated at 21 years of age, considering social and cultural factors *(5)*. However, pap smears have limitations, including subjective interpretation, variable sensitivity, and a shortage of trained cytotechnologists *(6)*.

Molecular HPV testing has emerged as a highly sensitive alternative due to its ability to detect high-risk HPV genotypes and associated cervical lesions, making it a valuable addition to cervical cancer screening *(7)*. Techniques such as polymerase chain reaction (PCR) and nucleic acid hybridization facilitate direct detection of HPV DNA in cervical samples, aiding risk stratification and clinical management. While pap smear remains widely used, PCR is considered the gold standard for HPV detection, as endorsed by the United States Preventive Services Task Force (USPSTF). High-risk HPV DNA detection enhances triage efficiency and optimizes patient management *(8)*.

Despite the proven efficacy of molecular HPV testing, its integration into Bahrain's national screening programme remains debated. The comparative effectiveness of pap smear and HPV PCR in identifying

cervical abnormalities among Bahraini women has not been fully explored. This study addressed this gap by assessing the concordance between these diagnostic tools, evaluating their strengths and limitations, and providing evidence-based insights to optimal cervical cancer screening strategies.

Methods

This comparative study analysed patient records from the Salmanyia Medical Complex Laboratory in Bahrain. A total of 320 cervical pap smear samples were collected from women attending the gynaecology department for routine screening or gynaecological concerns.

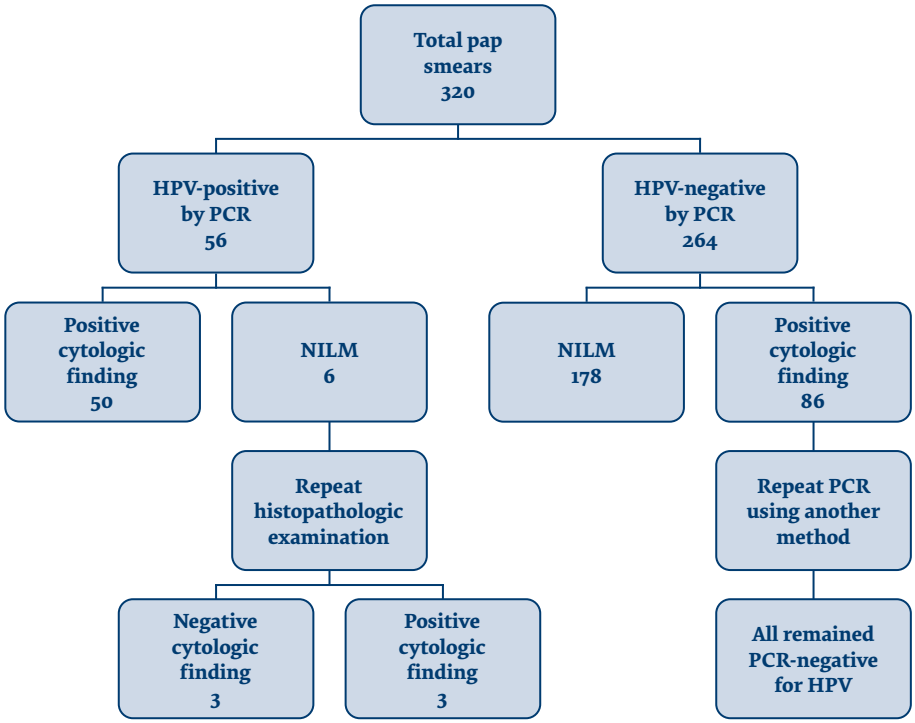
Cervical cytology was performed using liquid-based cytology. Samples were randomly selected for inclusion between May and October 2023 *(9)*. Molecular HPV PCR was conducted using the Sansure real-time high-risk HPV PCR assay which detects 23 high-risk HPV genotypes, including HPV 16 and 18, while grouping other genotypes under "others." Correlations between cytologic results and histopathological diagnoses were examined (Figure 1).

Cases with discordant results – negative pap smear but HPV-positive PCR findings – underwent histopathological re-evaluation. Two independent pathologists conducted blinded histopathologic examinations to minimise bias. Similarly, HPV-negative samples with abnormal cytology were re-evaluated using an alternative molecular PCR assay (Cepheid's GeneXpert HPV assay) to confirm findings *(10)*.

The study included all cervical cytology and HPV detection tests performed during the study period. Cases with incomplete or missing data or a history of cervical cancer or previous treatment for cervical abnormalities were excluded. Data collected included patient demographics, cytological findings, molecular HPV detection results (including genotypes) and, where available, histopathologic outcomes.

The study's statistical power was estimated using the Power and Precision V3 software. Categorical variables were compared using the chi-square (when all expected frequencies were ≥ 5) or Fisher's exact test (when any expected frequency was < 5). Continuous variables, such as age, were analysed using independent t-tests. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated for pap smears, using HPV PCR as the gold standard. Subgroup analyses was used to examine the influence of age, HPV genotype and cytologic findings on diagnostic accuracy. $P < 0.05$ was considered statistically significant *(11)*. SPSS version 19 was used for all statistical analyses.

This study adhered to the Declaration of Helsinki and was approved by the Institutional Review Board (IRB) of Salmaniya Medical Complex. Patient confidentiality was maintained through data anonymization. Informed consent was not required, because no personally identifiable data were used.



NILM = negative for intraepithelial lesion or malignancy; HPV = human papilloma virus

Figure 1. Study design: distribution of pap smear results, HPV detection and cytologic findings

Results

A total of 320 samples from 320 women were included, with a mean age of 43.7 ± 11.6 years, median age of 43 years, and age range 21 to 82 years. Among these, 56 (17.5%) tested positive for HPV by PCR, while 264 (82.5%) tested negative. The PCR-negative group was significantly larger ($P < 0.0001$), but mean age differences were not statistically significant ($P > 0.05$). The proportions of PCR-positive and PCR-negative cases are presented descriptively to provide context for the study population. Statistical comparisons, such as chi-square or Fisher's exact tests, were applied to examine associations between categorical variables (e.g. cytologic findings and PCR status), rather than determining the significance of the overall proportions of PCR-positive versus PCR-negative cases (Table 1).

Among 320 cervical smears, 57.5% were NILM, while 42.5% showed abnormalities, the most frequent being atypical squamous cells of undetermined significance (ASCUS) (22.0%), atypical glandular cells (AGC) (8.1%) and low grade squamous intraepithelial lesion (LSIL) (13.2%) (Image 1). Less common findings included atypical squamous cells (ASC-H) (3.8%), high-grade pre-cancerous condition (HSIL) (1.5%) (Image 2), squamous cell carcinoma (SCC) (0.6%), adenocarcinoma (0.3%), malignant sarcoma (0.3%) and atypical endocervical cells in favour of neoplasia (0.3%).

Among the HPV-positive women, the mean age was 40.7 ± 13.1 years, ranging from 23 to 82 years, with a median age of 37.5 years. We further stratified this group into 2 age subgroups: 52.0% were younger than 40 years and 48.0% were 40 years or older. Of the HPV-positive samples, 14 (25.0%) were positive for HPV 16, 2 (3.6%) were positive for HPV 16 in addition to other genotypes, and 1 (1.8%) was positive for HPV 18 in addition to other genotypes. Forty-two (75.0%) were positive for other HPV genotypes. Genotype prevalence did not significantly differ by age, and non-16/18 HPV genotypes were the most common. Cytologic examination of the HPV-positive cases showed NILM in 6 (11.0%) samples, AGC in 2 (3.6%), ASCUS in 22 (39.0%), ASC-H in 6 (11.0%), LSIL in 15 (27.0%), HSIL in 3 (5.4%), and SCC in 2 (3.6%). Of the 6 HPV-positive NILM smears, histopathology confirmed abnormalities in 2 (33.3%).

The 264 HPV-negative cases (82.5% of total samples) had a mean age of 44.3 ± 11.1 years, a median age of 44 years and age range of 21 to 75 years. Cytologic findings in this group included NILM in 178 (67.4%) cases, AGC

Table 1. Characteristics of the study groups based on HPV PCR results and cytological findings

Category	Total samples	HPV-positive cases	HPV-negative cases	*P1*	NILM cases	Positive cytology smears	*P2*
Number (%)	320 (100%)	56 (17.5%)	264 (82.5%)	< 0.0001*	184 (57.5%)	136 (42.5%)	< 0.0001*
Age (years)							
Mean ± SD	43.7 ± 11.6	40.7 ± 13.1	44.3 ± 11.1	< 0.05*	43.3 ± 11.4	44.2 ± 11.8	> 0.05
Median	43	37.5	44		42	44	
Range	21–82	23–82	21–75		21–75	23–82	
≥70	10.3 (7)	16.9 (119)	11.5 (21)				
HPV genotypes detected							
HPV 16	16 (5.0%)	14 (25.0%)	–		6 (3.3%)	10 (7.4%)	< 0.001*
HPV 18	1 (0.3%)	1 (1.8%)	–		–	1 (0.7%)	
Other	42 (13.1%)	42 (75.0%)	–		33 (18.0%)	9 (6.6%)	< 0.001*
Cytologic type							
NILM	184 (57.5%)	6 (11.0%)	178 (67.4%)	< 0.0001*	184 (100%)	–	
AGC	26 (8.1%)	2 (3.6%)	24 (9.1%)	> 0.05	–	26 (19.1%)	
ASCUS	70 (22.0%)	22 (39.0%)	48 (18.2%)	< 0.001*	–	70 (51.5%)	
ASC-H	12 (3.8%)	6 (11.0%)	6 (2.3%)	< 0.01*	–	12 (8.8%)	
LSIL	18 (5.6%)	15 (27.0%)	3 (1.1%)	< 0.0001*	–	18 (13.2%)	
HSIL	5 (1.5%)	3 (5.4%)	2 (0.8%)	< 0.05*	–	5 (3.7%)	
SCC	2 (0.6%)	2 (3.6%)	–		–	2 (1.5%)	
Adenocarcinoma	1 (0.3%)	–	1 (0.4%)		–	1 (0.75%)	
Malignant sarcoma	1 (0.3%)	–	1 (0.4%)		–	1 (0.75%)	
Atypical endocervical cells	1 (0.3%)	–	1 (0.4%)		–	1 (0.75%)	

NILM = negative for intraepithelial lesion or malignancy; AGC = abnormal glandular cells; ASCUS = atypical squamous cells of undetermined significance; ASC-H = atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion; LSIL = Low-Grade squamous intraepithelial lesion; HSIL = high-grade squamous intraepithelial lesion; SCC = squamous cell carcinoma

*P values represent statistical comparisons between groups: P1: Differences between HPV-positive and HPV-negative cases; P2: Differences between NILM and abnormal cytology groups. * Significant results (P < 0.05)*

in 24 (9.1%), ASCUS in 48 (18.2%), ASC-H in 6 (2.3%), LSIL in 3 (1.1%), HSIL in 2 (0.8%), vaginal adenocarcinoma in 1 (0.4%), malignant sarcoma in 1 (0.4%), and atypical endocervical cells in favour of neoplasia in 1 (0.4%). Comparison of cytologic findings between the 2 groups showed that the HPV-positive group had significantly higher rates of ASCUS, ASC-H, LSIL and HSIL, but fewer NILM cases than the HPV-negative group. No significant differences were observed in AGC prevalence. The HPV-positive group had one case of SCC, while the HPV-negative group had one case each of adenocarcinoma, malignant sarcoma, and atypical endocervical cells in favour of neoplasia. HPV-PCR demonstrated a sensitivity of 95.5% (95% CI: 87.5–98.9%), a specificity of 67.4%, a PPV of 94.6% and an NPV of 96.9%.

Cytology results of the pap smears showed 184 (57.5%) were NILM, while 136 (42.5%) showed significant cytologic changes. The size difference between these groups was statistically significant ($P < 0.0001$), but no significant differences were observed in mean age ($P > 0.05$). The NILM group had a mean age of 43.3 ± 11.4 years, a median age of 42 years and age range 21 to 75 years. Among the 184 NILM smears, 6 (3.3%) were HPV-positive by PCR due to strains other than HPV 16 or 18. Three of these 6 cases (50.0%) showed significant histopathologic changes upon re-evaluation. Also, 178 (96.7%) of the NILM smears were HPV-negative and showed no significant histopathologic changes.

The group with abnormal cytologic findings had a mean age of 44.2 ± 11.8 years, a median age of 42 years, and age range 23 to 82 years. Among these smears, 50 (36.8%) were HPV-positive, with 14 (10.3%) positive for HPV 16, 2 (1.5%) positive for HPV 16 in addition to other genotypes, and 1 (0.7%) positive for HPV 18 in addition to other genotypes. Thirty-three (24.3%) samples were positive for other HPV genotypes. The cytologic findings included 26 cases (19.1%) with AGC, 70 (51.5%) with ASCUS, 12 (8.8%) with ASC-H, 18 (13.2%) with LSIL, 5 (3.7%) with HSIL, 2 (1.5%) with SCC, 1 (0.75%) with vaginal adenocarcinoma, 1 (0.75%) with malignant sarcoma, and 1 (0.75%) with atypical endocervical cells in favour of neoplasia.

Discussion

Early detection of cervical cancer is critical, because cervical cancer remains a major cause of cancer-related morbidity and mortality among women globally, with an estimated 604 000 new cases reported annually *(12)*. In Bahrain, cervical cancer is the 8th most common cancer among women aged 15–44 years, with 21 new diagnoses and 12 related deaths each year *(13)*. These figures underscore the importance of effective screening tools.

This study aimed to compare the effectiveness of pap smear and HPV detection by PCR in identifying precancerous and cancerous lesions in women from Bahrain. The results provide valuable insights to the link between PCR testing for HPV and cytologic findings in cervical smear.

Our findings indicate that PCR testing has a high sensitivity and positive predictive value for detecting histopathologic lesions in cervical samples, with a sensitivity of 95.5% and a positive predictive value of 94.6%. Certain cases had discordant results – negative pap smear but positive by PCR – indicating that PCR may detect HPV infections even when cytologic findings fail to do so, as previously reported in the literature. The moderate specificity of PCR (67.4%) suggests that while it is highly sensitive, it may detect transient infections or non-pathogenic HPV genotypes, leading to false positives.

Although molecular methods like PCR are typically highly sensitive and specific, several factors may have contributed to the reduced specificity observed in our findings. First, PCR's sensitivity allows it to detect even transient HPV infections, which are common in younger populations and may resolve without leading to significant cervical lesions *(14)*. This ability to identify transient infections likely contributed to cases where PCR results were positive without accompanying cytologic abnormalities *(15)*. Another contributing factor is the potential for cross-reactivity, because certain PCR assays, including the Sansure real-time high-risk HPV PCR assay used in our study, may occasionally react with non-HPV DNA sequences or environmental contaminants despite stringent contamination controls *(16)*. Although the kit detected 23 high-risk HPV genotypes, it only provided genotype-specific identification for HPV 16 and 18, grouping all other genotypes as "other high-risk types." This lack of precision may have influenced our specificity findings, as some genotypes were more likely to be transient and non-pathogenic. Variations in sample collection and DNA quality may also have played a role; samples with compromised quality (e.g. insufficient cellular material) may yield HPV DNA detection even in the absence of cellular abnormalities *(17)*. Our findings are consistent with previous studies that reported specificity variations, particularly in populations with high transient infection rates or broad-spectrum screening *(18-20)*. Thus, we recommend that HPV PCR results be interpreted alongside cytologic findings and patient history to improve diagnostic accuracy.

Importantly, our findings revealed that most HPV-positive cases detected by PCR were attributed to strains other than HPV 16 and 18. This underscores the importance of comprehensive HPV testing that covers a broad spectrum of high-risk HPV genotypes beyond the commonly targeted strains. This is in line with the findings of Alnoaimi et al, who reported that about 62–73% of HPV genotypes detected in abnormal pap smears were not caused by genotype 16 or 18, especially in women younger than 40 years *(21)*.

We did not find a significant effect of age on genotype prevalence in this study. We found that HPV genotype 16 was identified in 28.5% of positive cases, which is consistent with findings by Zheng et al. *(22)*. A significant limitation of our study was that we identified genotypes other than 16 and 18 as "others". Further research is

needed to determine the precise prevalence and clinical significance of individual genotypes. Multiple factors may influence genotype distribution, such as age, gender, ethnicity, socioeconomic status, sexual behaviour, HPV vaccination status, immune status, geographical location, smoking, and other co-factors, along with screening and diagnostic practices that influence viral detection and reporting *(23)*.

Our study highlighted a critical observation regarding the subset of NILM smears that tested positive for HPV by PCR. Upon re-evaluation through histopathologic examination, a significant proportion of these cases exhibited underlying cervical lesions. Specifically, out of the 6 patients with NILM and positive HPV-PCR, histopathologic examination confirmed the presence of significant cervical lesions in 3 cases. This underscores the potential usefulness of PCR testing in identifying HPV infection, even in cases with seemingly normal cytologic findings and highlights the potential limitations of relying solely on cytology. Our finding agrees with the work of Matah et al, who found that HPV testing provides more precise cervical cancer surveillance strategies than pap smear *(24)*. A recent Canadian study recommended transitioning from pap testing to HPV testing in Canadian primary cervical cancer screening due to its higher sensitivity, cost-effectiveness, and safety *(25)*. However, we recommend combining pap smear with HPV testing to increase the sensitivity and decrease the risk of false positive results.

Our analysis of cytologic findings in cervical smears revealed a diverse range of abnormalities, including NILM, AGC, ASCUS, ASC-H, LSIL, HSIL, SCC, adenocarcinoma, malignant sarcoma, and atypical endocervical cells in favour of neoplasia. Notably, the HPV-positive group exhibited significantly higher frequencies of smears with ASCUS, ASC-H, LSIL (Images 1–4), and HSIL (Images 5–8) than the HPV-negative group. These findings agree with the work of Faqih et al, who found an increased risk of

Case 1: Low-grade squamous intraepithelial lesion with follow-up findings
These images show the cytologic and histologic features typical of a low-grade lesion, including early changes visible on the pap smear, with biopsy confirmation and immunohistochemical markers supporting the presence of HPV-related changes.

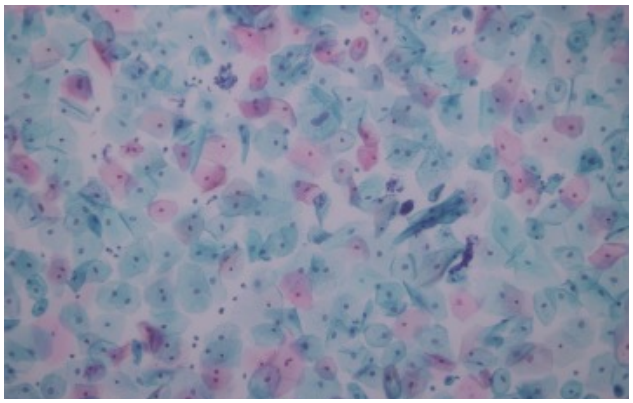


Image 1. Pap smear from a 53-year-old woman showing features of a low-grade squamous intraepithelial lesion (LSIL). The image highlights large cell size, dark chromatin, binucleation and a well-defined cytoplasm (Papanicolaou stain, 400x)

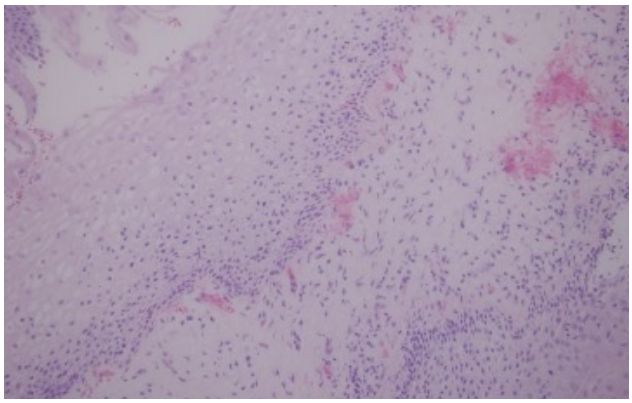


Image 2. Cervical biopsy from the same patient, showing cervical intraepithelial neoplasia I (CIN I) with nuclear atypia involving the lower one-third of the squamous lining (Hematoxylin and eosin stain, 400x)

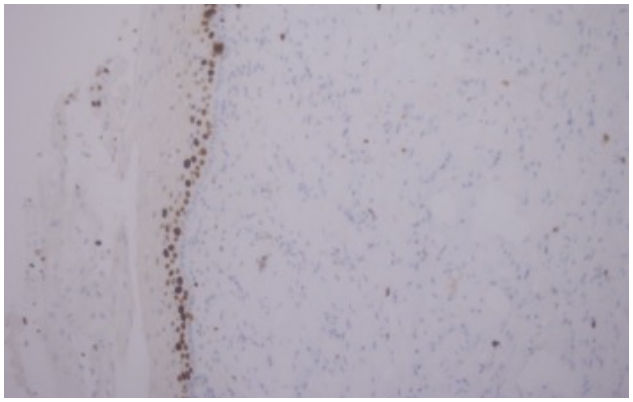


Image 3. Cervical biopsy of the same patient, showing cervical intraepithelial neoplasia I (CIN I) with nuclear atypia involving the lower one-third of the squamous lining (ki.67 stain, 400x)

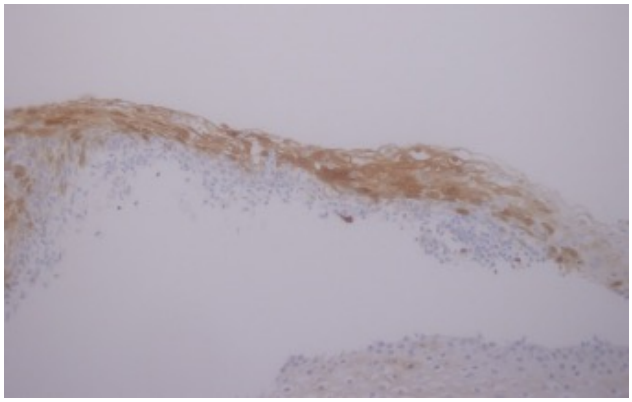


Image 4. Strong diffuse nuclear and cytoplasmic staining for p16, which is associated with HPV-induced changes (400x)

ASC-H, HSIL, and LSIL in patients who tested positive for genotype 16, increased risk of LSIL in patients who tested positive for genotypes 18/45, and an increased risk of ASCUS and LSIL in patients who tested positive for other high-risk HPV genotypes *(26)*. Kim et al found that increasing severity of cervical cytology is linked with infection with certain HPV genotypes *(27)*. This suggests that PCR testing may be particularly useful for identifying cases with more advanced cervical cytologic abnormalities and in stratifying the risk of cervical cancer *(28)*.

Interestingly, while the HPV-negative group had a higher proportion of smears with NILM, it also included cases with adenocarcinoma, malignant sarcoma, and atypical endocervical cells in favour of neoplasia. This highlights the importance of considering both PCR and cytologic findings in assessing cervical health. However, it is important to acknowledge the limitations of PCR testing, including the potential for false-positive results and the need for careful interpretation in the context of clinical findings. Despite its high sensitivity for detecting HPV infection, PCR testing may yield false-positive results, necessitating confirmatory testing, and correlation with clinical and histopathological findings.

Therefore, in interpreting the results of HPV PCR testing and cervical pap smear analyses, it is essential to recognize the potential for both false positive and false negative outcomes. False positives may occur due to factors such as contamination during sample collection or processing, cross-reactivity with non-target DNA, or detection of transient HPV infections that may not progress to cervical abnormalities *(29,30)*. Conversely,

Case 2: High-grade squamous intraepithelial lesion (HSIL) with follow-up findings
These images represent the cellular and tissue-level changes associated with high-risk HPV infection, illustrating the progression from cytologic findings to histopathologic confirmation. The IHC staining (Ki-67 and p16) visually emphasizes the proliferative activity and HPV association in high-grade lesions.

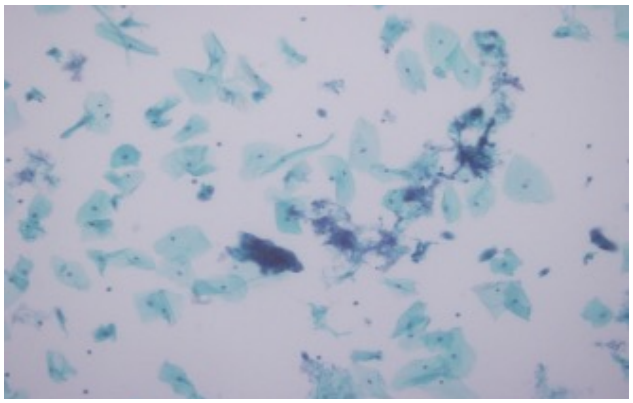


Image 5. Pap smear image from a 52-year-old HPV-positive woman showing high-grade squamous intraepithelial lesion (HSIL), with hyperchromatic crowded cell groups, clearly marked by an arrow (Papanicolaou stain, 400x)

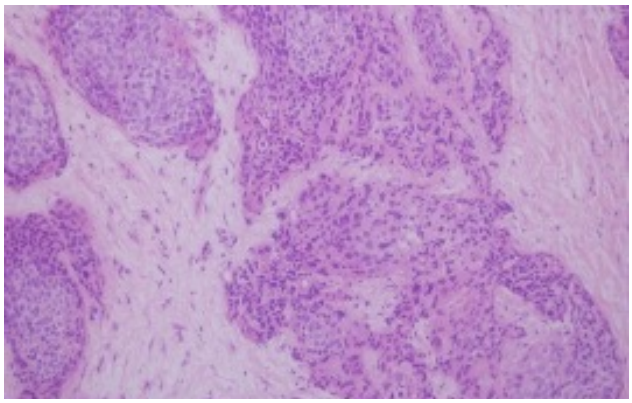


Image 6. Follow-up cervical biopsy revealing cervical intraepithelial neoplasia-3 (CIN-3), characterised by full-thickness nuclear atypia (haematoxylin and eosin stain, 400x), alongside invasive squamous cell carcinoma. There are clusters of epithelial cells with hyperchromatic nuclei and irregular architecture, suggesting a high-grade squamous intraepithelial lesion (HSIL)

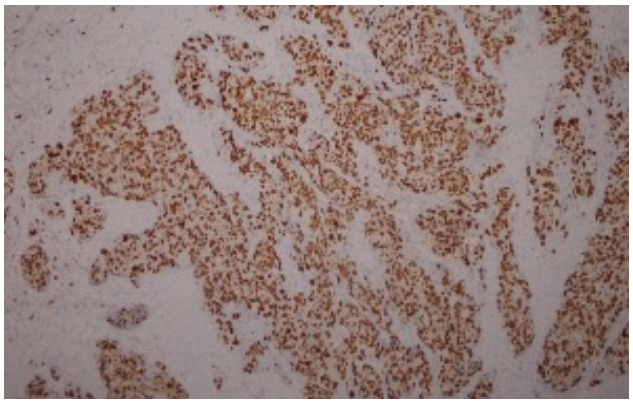


Image 7. Ki-67 immunohistochemical stain showing a high proliferation index, indicating active cellular turnover (200x)

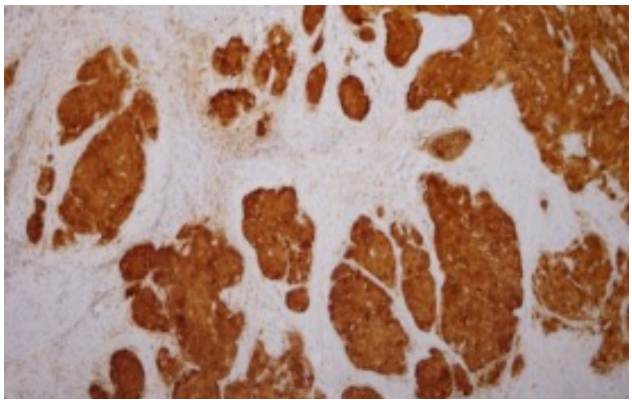


Image 8. Strong and diffuse nuclear and cytoplasmic staining for p16, an indicator of HPV-associated transformation, which supports the diagnosis of HSIL and CIN-3 (200x)

false negatives can arise from inadequate sampling, low viral load below the detection threshold, or genetic variations that affect primer binding in PCR assays *(31)*. Therefore, caution is needed in interpreting results, and consideration should be given to corroborating findings with clinical history, cytologic features, and histopathologic examinations. Interpretation of pap smear results should integrate additional factors such as patient age, reproductive history, immunologic status, and HPV vaccination status, which may influence the likelihood of cervical dysplasia or cancer development *(32)*.

In addition to our primary findings, it is essential to consider co-morbid and background factors that may influence HPV infection risk and progression to cervical abnormalities in our study population *(33)*. Age is a significant factor, because younger women often experience transient HPV infections that may resolve without intervention. In contrast, persistent infections – which carry a higher risk of progression to dysplasia or cancer – are more common in older age groups *(34)*. The immune status also plays a crucial role; immunocompromised individuals, such as those with HIV or autoimmune conditions, are more susceptible to persistent HPV infections and, thus, at greater risk for cervical pathology *(35)*. Socioeconomic factors are equally impactful, as individuals from lower socio-economic backgrounds may face reduced access to regular screening and HPV vaccination, heightening their risk of undetected or untreated infections *(36)*. Lifestyle factors, particularly smoking, can also compromise local immune responses in the cervix, potentially facilitating HPV persistence and progression *(37)*. Consideration of these co-factors enables a more nuanced understanding of HPV epidemiology and supports the development of population-specific prevention strategies.

Study limitations

Despite the valuable insights gained from this study, several limitations should be acknowledged. First, the study was conducted at a single centre, which may limit the generalisability of the findings to broader populations. Future multicentre studies involving more diverse patient demographics are needed to validate the results and improve the external validity of the study.

Second, while PCR testing for HPV detection is highly sensitive, it is also susceptible to false-positive results. Although we attempted to mitigate this limitation by confirming positive PCR results through histopathologic examination, the possibility of false-positive results cannot be entirely excluded. Therefore, cautious interpretation of PCR results is essential and further validation studies are recommended to assess the accuracy of PCR testing across different clinic settings.

Third, our study focused primarily on the association between HPV detection by PCR and cytologic findings in cervical smears. While PCR testing offers valuable information on HPV infection status, it does not capture other important factors that may influence cervical cancer risk, such as viral load and persistence. Future studies incorporating additional HPV testing modalities, such as viral load quantification and genotyping, would provide a more comprehensive understanding of HPV-related cervical pathology.

Fourth, histopathologic confirmation of cervical abnormalities was not available for all patients, which may have affected the accuracy of diagnostic comparisons. Another limitation is the absence of data on HPV vaccination status, which may have influenced both HPV detection rates and cytological outcomes. Given the increasing prevalence of HPV vaccination among many populations, future studies should explore its impact on HPV prevalence and cervical pathology to inform screening strategies and vaccination policies.

Despite these limitations, the study contributes meaningful insights to the complex interplay between HPV detection by PCR, cytologic findings in cervical smears, and cervical pathology. Addressing the identified limitations through further research will be critical to enhancing our understanding of HPV-related cervical disease and improving clinical management strategies for populations at risk.

Recommendations

To enhance cervical cancer screening in Bahrain, a comprehensive approach integrating HPV PCR testing alongside pap smear screening is recommended. This combined strategy can improve detection rates, particularly in cases with negative or inconclusive cytologic findings. Combining molecular diagnostics with cytologic examination can enhance early detection and improve patient management based on clinical history and symptoms, because PCR may detect transient infections that cytology may miss.

Future screening programmes should use broad-spectrum HPV tests to detect a wide range of high-risk genotypes beyond HPV 16 and 18. Tailoring screening protocols based on age groups and implementing educational campaigns to raise awareness about screening benefits and limitations are essential. Quality assurance measures in HPV PCR testing laboratories and clear management guidelines for patients with positive results, including normal cytology, are crucial. Continued research into HPV epidemiology and the establishment of robust surveillance programmes will inform evidence-based screening and vaccination strategies, ultimately reducing the burden of cervical cancer in Bahrain and promoting women's health.

Conclusion

This study highlights the significance of integrating HPV PCR testing with conventional pap smear screening for effective cervical cancer detection in Bahraini women. The high sensitivity of PCR testing for HPV highlights its value as a tool for identifying cervical lesions, particularly

in cases with seemingly normal cytologic findings. However, the moderate specificity of PCR testing requires careful consideration, emphasizing the need for integrating clinic and histopathologic assessments to ensure accurate diagnosis.

These findings support integrating broad-spectrum HPV testing into screening protocols, particularly for cases with inconclusive cytology, to enhance timely detection and risk stratification. By adopting a holistic approach to cervical cancer screening and leveraging advances in molecular diagnostics, health care providers can improve early detection rates and ultimately reduce the burden of cervical cancer in Bahrain and other similar settings.

Acknowledgements

We thank the anonymous reviewers and editors for their valuable suggestions and constructive feedback, which significantly improved the quality of this manuscript. We also acknowledge the Salmaniya Medical Complex Laboratory and the Department of Gynaecology for their support in data collection. Special thanks to all participants and our colleagues for their contributions and insights.

Funding: None.

Conflict of interest: None declared.

Étude comparative du frottis vaginal et des tests d'amplification génique pour le dépistage du papillomavirus humain à Bahreïn

Résumé

Contexte : Le cancer du col de l'utérus demeure une préoccupation de santé mondiale, en particulier dans les pays à revenu faible ou intermédiaire (auxquels Bahreïn appartient), où l'infection par le virus du papillomavirus humain (VPH) en est la principale cause.

Objectif : Comparer la précision diagnostique de l'examen cytologique (frottis vaginal) et de la détection moléculaire du VPH à l'aide des tests d'amplification génique (PCR) dans l'identification des anomalies cervicales chez les femmes à Bahreïn.

Méthodes : Nous avons analysé rétrospectivement 320 échantillons de frottis provenant du laboratoire du Complexe médical Salmaniya. Les résultats cytologiques ont été comparés aux résultats des tests PCR pour le VPH, en prenant la PCR comme référence. La sensibilité, la spécificité ainsi que les valeurs prédictives positives et négatives ont été calculées. Les analyses statistiques comprenaient des tests du chi-carré et des tests exacts de Fisher.

Résultats : Sur ces 320 échantillons, 56 (17,5 %) étaient positifs et 264 (82,5 %) négatifs au VPH. La PCR a montré une sensibilité plus élevée (95,5 %) mais une spécificité modérée réduite (67,4 %), avec une valeur prédictive positive pour la détection des lésions histopathologiques de 94,6 % et une valeur prédictive négative de 96,9 %. Les frottis vaginaux ont permis d'identifier 57,5 % des échantillons comme négatifs pour les lésions intraépithéliales ou les tumeurs malignes et 42,5 % pour les anomalies. Les cas positifs au VPH présentaient des taux cytologiques anormaux plus élevés et des lésions confirmées par histopathologie pour certains d'entre eux, malgré des résultats cytologiques normaux.

Conclusion : L'association de la PCR pour le VPH à un frottis vaginal améliore la détection du cancer du col de l'utérus. Afin de réduire la charge de morbidité, il est recommandé de mettre en place des programmes de dépistage adaptés en fonction des facteurs de risque individuels.

دراسة مقارنة لاختبارات لطاخة بابا نيكولاو وتفاعل البوليميراز المتسلسل لتحري فيروس الورم الحليمي البشري في البحرين

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الخلاصة

الخلفية: لا يزال سرطان عنق الرحم شاغلاً صحيًّا عالميًّا، لا سيما في البلدان ذات الدخل المنخفض والمتوسط، ومنها البحرين، إذ تمثل عدوى فيروس الورم الحليمي البشري السبب الرئيسي للإصابة به.

الأهداف: هدفت هذه الدراسة الى مقارنة الدقة التشخيصية لفحص خلايا عنق الرحم (لطاخة بابا نيكولاو) والكشف الجزيئي عن فيروس الورم الحليمي البشري باستخدام تفاعل البوليميراز المتسلسل في تحديد تشوهات عنق الرحم بين النساء في البحرين.

طرق البحث: حللنا بأثر رجعي 320 عينة من مسحات عنق الرحم (لطاخة بابا نيكولاو) من مختبر مجمع السلمانية الطبي. وقورنت النتائج الخلوية بنتائج تفاعل البوليميراز المتسلسل لفيروس الورم الحليمي البشري، باستخدام المقياس الدقيق اختبار تفاعل البوليميراز المتسلسل.

وحُسبت الحساسية والنوعية والقيمة التنبؤية الإيجابية والقيمة التنبؤية السلبية. وشملت التحليلات الإحصائية اختبار مربع كاي واختبار فيشر الدقيق.

النتائج: من بين 320 عينة، كانت 56 عينة (17.5%) إيجابية لفيروس الورم الحليمي البشري، و264 عينة (82.5%) سلبية لفيروس الورم الحليمي البشري. وأظهر تفاعل البوليمراز المتسلسل حساسية أعلى (95.5%)، ولكن نوعية معتدلة أقل (67.4%)، وبلغت القيمة التنبؤية الإيجابية للكشف عن آفات الأنسجة والقيمة التنبؤية السلبية 94.6% و96.9%، على التوالي. وأظهرت مسحات بابا نيكولاو أن 57.5% من العينات كانت سلبية للآفات داخل الظهارة أو الأورام الخبيثة، وأن 42.5% من العينات كانت بها تشوهات. وكانت معدلات الخلايا غير الطبيعية أعلى في الحالات الإيجابية لفيروس الورم الحليمي البشري، وأكد فحص مرضيات الأنسجة وجود آفات في بعض الحالات الإيجابية لفيروس الورم الحليمي البشري على الرغم من نتائج الخلايا الطبيعية.

الاستنتاجات: من شأن الجمع بين تفاعل البوليميراز المتسلسل لفيروس الورم الحليمي البشري ولطاخة بابا نيكولاو أن يعزز اكتشاف سرطان عنق الرحم. ويُوصى بوضع برامج تحرٍّ مُصممة على أساس عوامل الخطر الفردية للحد من عبيء المرض.

References

1. Bogdanova A, Andrawos C, Constantinou C. Cervical cancer, geographical inequalities, prevention and barriers in resource depleted countries. Oncol Lett. 2022;23(4):113. DOI:10.3892/ol.2022.13233
2. Jassim G, Obeid A, Al Nasheet HA. Knowledge, attitudes, and practices regarding cervical cancer and screening among women visiting primary health care centres in Bahrain. BMC Public Health. 2018;18(1):128. DOI:10.1186/s12889-018-5023-7
3. Johnson CA, James D, Marzan A, Armaos M. Cervical cancer: an overview of pathophysiology and management. Semin Oncol Nurs. 2019;35(2):166–174. DOI: 10.1016/j.soncn.2019.02.003
4. Mishra GA, Pimple SA, Shastri SS. An overview of prevention and early detection of cervical cancers. Indian J Med Paediatr Oncol. 2011;32(3):125–132. DOI:10.4103/0971-5851.92808
5. ACOG Committee Opinion No. 463. Cervical cancer in adolescents: screening, evaluation, and management. Obstet Gynecol. 2010;116(2 Pt 1):469–472. DOI:10.1097/AOG.0b013e3181eeb30f
6. Nikumbh DB, Nikumbh RD, Kanthikar SN. Limitations of cytological cervical cancer screening (Papanicolaou test) regarding technical and cultural aspects in rural India. South Asian J Cancer. 2016;5(2):79. DOI: 10.4103/2278-330X.181646
7. Gradíssimo A, Burk RD. Molecular tests potentially improving HPV screening and genotyping for cervical cancer prevention. Expert Rev Mol Diagn. 2017;17(4):379–391. DOI: 10.1080/14737159.2017.1293525
8. Tao X, Austin RM, Yu T, Zhong F, Zhou X, Cong Q, et al. Risk stratification for cervical neoplasia using extended high-risk HPV genotyping in women with ASC–US cytology: a large retrospective study from China. Cancer Cytopathol. 2022;130(4):248-–258. DOI:10.1002/cncy.22536
9. Sharma J, Toi PCh, Siddaraju N, Sundareshan M, Habeebullah S. A comparative analysis of conventional and SurePath liquid-based cervicovaginal cytology: a study of 140 cases. J Cytol. 2016;33(2):80–4. DOI:10.4103/0970-9371.182525
10. Salvia PN, Bergo SM, Bonesso-Sabadini PI, Tagliarini EB, Hackel C, Andrade LA. Correlation between histological criteria and human papillomavirus presence based on PCR assay in cervical biopsies. Int J Gynecol Cancer. 2004;14(1):126–32. DOI:10.1111/j.1048-891x.2004.014030.x
11. Suresh K, Chandrashekara S. Sample size estimation and power analysis for clinical research studies. J Hum Reprod Sci. 2012;5(1):7–13. DOI:10.4103/0974-1208.97779
12. 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(3):209–249. DOI:10.3322/caac.21660
13. Husain Y, Alalwan A, Al-Musawi Z, Abdulla G, Hasan K, Jassim G. Knowledge towards human papillomavirus (HPV) infection and attitude towards its vaccine in Bahrain: cross-sectional study. BMJ Open. 2019;9(9):e031017. DOI:10.1136/bmjopen-2019-031017
14. García DA, Cid-Arregui A, Schmitt M, Castillo M, Briceño I, Aristizábal FA. Highly sensitive detection and genotyping of HPV by PCR multiplex and Luminex technology in a cohort of Colombian women with abnormal cytology. Open Virol J. 2011;5:70–9. DOI:10.2174/1874357901105010070
15. Cameron JE, Dennis DC, Herrel NR, Chapple AG, Hagensee ME. Risk of abnormal cervical cytology in HIV-infected women testing positive for both human papillomavirus and Epstein-Barr virus in genital tract specimens. Cancer Causes Control. 2020;31(4):365–375. DOI:10.1007/s10552-020-01287-z
16. Preisler S, Rebolj M, Ejegod DM, Lynge E, Rygaard C, Bonde J. Cross-reactivity profiles of hybrid capture II, cobas, and APTIMA human papillomavirus assays: split-sample study. BMC Cancer. 2016;16:510. DOI:10.1186/s12885-016-2518-4
17. Vashisht V, Vashisht A, Mondal AK, Farmaha J, Alptekin A, Singh H, Ahluwalia P, Srinivas A, Kolhe R. Genomics for emerging pathogen identification and monitoring: prospects and obstacles. BioMedInformatics. 2023;3(4):1145–1177. DOI:10.3390/biomedinformatics3040069

18. Benevolo M, Vocaturo A, Caraceni D, French D, Rosini S, Zappacosta R, et al. Sensitivity, specificity, and clinical value of human papillomavirus (HPV) E6/E7 mRNA assay as a triage test for cervical cytology and HPV DNA test. J Clin Microbiol. 2011;49(7):2643-50. DOI: 10.1128/JCM.02570-10

19. Fleider LA, de Los Ángeles Tinnirello, M., Gómez Cherey F, García MG, Cardinal LH, García Kamermann F, et al. High sensitivity and specificity rates of cobas HPV test as a primary screening test for cervical intraepithelial lesions in a real-world setting. PLoS One. 2023;18(2):e0279728. DOI:10.1371/journal.pone.0279728

20. Lv N, Zhao Y, Song Y, Ji M, Zhou Y. Development of a sensitive droplet digital PCR according to the HPV infection specificity in Chinese population. BMC Cancer. 2023;23(1):1022. DOI:10.1186/s12885-023-11529-3

21. Alnoaimi AM, Feda TH, Azam M, Syed S, Hafiz K, Saad HM, et al. Prevalence and correlation between high-risk HPV genotypes and Pap smear findings in Bahrain: a retrospective approach. Int J Biomed. 2024;14(1):52–58. DOI:10.21103/Article14(1)_OA7

22. Zheng L, Chen S, Liu W, Qi J, Li K. Human papillomavirus prevalence and genotype distribution in Liaocheng men between 2016 and 2022. J Med Virol. 2024;96(1):e29360. DOI:10.1002/jmv.29360

23. Yang J, Wang W, Wang Z, Wang Z, Wang Y, Wang J, Zhao W, et al. Prevalence, genotype distribution and risk factors of cervical HPV infection in Yangqu, China: a population-based survey of 10086 women. Hum Vaccin Immunother. 2020;16(7):1645–1652. DOI: 10.1080/21645515.2019.1689743

24. Matah M, Sareen S. Detection of HPV by PCR – a novel step in the prevention of cancer cervix. J Obstet Gynaecol India. 2012;62(2):188–91. DOI: 10.1007/s13224-012-0167-3.

25. Delpero E, Selk A. Shifting from cytology to HPV testing for cervical cancer screening in Canada. CMAJ. 2022;194(17):E613–E615. DOI:10.1503/cmaj.211568

26. Faqih L, Alzamil L, Aldawood E, Alharbi S, Muzzaffar M, Moqnas A, Almajed H, Alghamdi A, Alotaibi M, Alhammadi S, Alwelaie Y. Prevalence of human papillomavirus infection and cervical abnormalities among women attending a tertiary care center in Saudi Arabia over 2 years. Trop Med Infect Dis. 2023;8(12):511. DOI:10.3390/tropicalmed8120511

27. Kim MJ, Kim JJ, Kim S. Type-specific prevalence of high-risk human papillomavirus by cervical cytology and age: data from the health check-ups of 7,014 Korean women. Obstet Gynecol Sci. 2013 Mar;56(2):110–20. DOI:10.5468/OGS.2013.56.2.110

28. Schiffman M, Wentzensen N, Wacholder S, Kinney W, Gage JC, Castle PE. Human papillomavirus testing in the prevention of cervical cancer. J Natl Cancer Inst. 2011;103(5):368–83. DOI: 10.1093/jnci/djq562

29. Núñez-Troconis J, Delgado M, González J, Velásquez J, Mindiola R, Whitby D, Conde B, Munroe DJ. Human papillomavirus false positive cytological diagnosis in low grade squamous intraepithelial lesion. Invest Clin. 2009;50(4):447–54. PMID: 20306718.

30. Schiffman M, de Sanjose S. False positive cervical HPV screening test results. Papillomavirus Res. 2019;7:184–187. DOI: 10.1016/j.pvr.2019.04.012

31. Macios A, Nowakowski A. False negative results in cervical cancer screening – risks, reasons and implications for clinical practice and public health. Diagnostics (Basel). 2022;12(6):1508. DOI:10.3390/diagnostics12061508

32. Choi S, Ismail A, Pappas-Gogos G, Boussios S. HPV and cervical cancer: a review of epidemiology and screening uptake in the UK. Pathogens. 2023;12(2):298. DOI:10.3390/pathogens12020298

33. Bowden SJ, Doulgeraki T, Bouras E, Markozannes G, Athanasiou A, Grout-Smith H, et al. Risk factors for human papillomavirus infection, cervical intraepithelial neoplasia and cervical cancer: an umbrella review and follow-up Mendelian randomization studies. BMC Med. 2023;21(1):274. DOI:10.1186/s12916-023-02965-w.

34. Rodríguez AC, Schiffman M, Herrero R, Hildesheim A, Bratti C, Sherman ME, et al Longitudinal study of human papillomavirus persistence and cervical intraepithelial neoplasia grade 2/3: critical role of duration of infection. J Natl Cancer Inst. 2010 Mar 3;102(5):315-24. DOI:10.1093/jnci/djq001

35. Hewavisenti RV, Arena J, Ahlenstiel CL, Sasson SC. Human papillomavirus in the setting of immunodeficiency: pathogenesis and the emergence of next-generation therapies to reduce the high associated cancer risk. Front Immunol. 2023;14:1112513. DOI:10.3389/fimmu.2023.1112513

36. Kurani S, MacLaughlin KL, Jacobson RM, St Sauver JL, Jenkins GD, Fan C, et al. Socioeconomic disadvantage and human papillomavirus (HPV) vaccination uptake. Vaccine. 2022;40(3):471–476. DOI:10.1016/j.vaccine.2021.12.003

37. Aguayo F, Muñoz JP, Perez-Dominguez F, Carrillo-Beltrán D, Oliva C, Calaf GM, et al. High-Risk human papillomavirus and tobacco smoke interactions in epithelial carcinogenesis. Cancers (Basel). 2020;12(8):2201. DOI: 10.3390/cancers12082201